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A Study on Factors Affecting Sleep during Pregnancy in Clinical Trials

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Division of Mathematics
Statistics

A Study on Factors Affecting Sleep during Pregnancy in Clinical Trials

Arts & Sciences Graduate Students
by
Zihan Yan

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Zihan Yan

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Abstract

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for Arts & Sciences Graduate Students

by

Zihan Yan

Master of Art in Statistics

Mathematics

Washington University in St. Louis, 2017

Professor Jeff Gill, Chair

Professor Edward Spitznagel, Co-Chair

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Regular sleep is required for sensory processing, learning, and brain plasticity. During pregnancy, poor sleep quality and dysregulation of hormones are all associated with increased risk for diseases like postpartum major depression[1]. Seventy-eight percent of pregnant women experience sleep problems at some point during pregnancy according to the National Sleep Foundation's 1998 Women and Sleep poll. Chronodisruption is a frequent sleep disturbance experienced by pregnant women that can be primary or due to co-morbid conditions[2]. For this reason, chronodisruption, which includes insomnia, is currently regarded as one of the most important factors determining pregnancy outcome. Therefore, the goal of this study is to find essential factors to model effects of midpoint time of sleep during different trimesters as a measure of sleep quality. In this study, we are going to focus on sleep changes during pregnancy. Our underlying hypothesis is that circadian rhythms in the mother, fetus, or both regulate timing

of parturition and, when disrupted, lead to preterm birth. I used linear regression models to address sleep changes during all three trimesters grouped by weekend and weekdays. Relationships between factors were investigated via correlation analysis. Interactions between melatonin/cortisol peak values and other factors such as sleep medication taken, vitamins taken, whether sleep was achieved within 30 min, and workload factors were explored. Other factors of interest such as race, having a paid job, and whether or not subjects had a night shift were investigated for overall midpoint sleep time as well as interactions with vitamins taken. Graphs were generated for models as well as for group comparisons. Correlation analysis, ANOVA, and linear regression methods were used to identify the most effective variable and to explain as much of the variance as possible.

Factors affecting sleep midpoint and sleep hormones such as workload, sleep medicine taken, and prior pregnancy were successfully selected for non-pregnancy and all three pregnant periods for regression models. Model selection was based on the best adjusted R-squared evaluation metric. More details are discussed.

Chapter 1: Introduction

1.1 Pregnant Sleep Problems

1.1.1 Overview of sleep and circadian rhythm disruption

Sleep and wakefulness cycles follow a circadian rhythm that is controlled primarily by the suprachiasmatic nucleus (SCN) of the hypothalamus, which is sensitive to both the light–dark cycle and hormones (i.e. melatonin and cortisol)[3]. Exposure to light at the right time helps keep the circadian clock on the correct time schedule. Exposure at the wrong time can shift sleep and wakefulness to undesired times. Circadian rhythm disturbances and sleep problems affect up to 90% of blind people, and demonstrate the importance of light to sleep/wake patterns. Circadian rhythms make people's desire for sleep strongest between midnight and dawn, and to a lesser extent in midafternoon. Such sleep/wake regulation makes circadian rhythms have a significant effect on body temperature, hormonal changes, blood glucose, and heart rate [4]. The classic phase markers for measuring the timing of a mammal's circadian rhythm are: melatonin secretion by the pineal gland, core body temperature minimum, and plasma level of cortisol[5].

Many factors can cause human circadian rhythm disruption. Aging has been associated with changes in the period and amplitude of circadian rhythms[6]. Environmental temperature, habits, seasonal variation and many other factors also contribute to circadian rhythm changes, and affect one's ability to respond to time cues and keep up with the demands of one's daily schedule. Furthermore, many sleep quite differently on workdays versus days off, a pattern which can lead to chronic circadian desynchronization[7].

The most common circadian sleep problem is jet lag [8]. This occurs when a person travels across many time zones. Symptoms related to jet lag include insomnia, daytime sleepiness,

indigestion, irritability and poor concentration. Another common problem is shift work disorder, which affects people who work night shifts or rotating shifts[8].People who are not able to fall asleep at a normal time at night are often diagnosed with delayed sleep phase disorder (DSP)[8]. This problem is more common in young adults than in other age groups. It can interfere with job performance or school and cause mental stress. Some others tend to get very sleepy in the early afternoon and go to bed much earlier than normal, and are thus diagnosed with advanced sleep phase disorder (ASP)[8]. This problem is more common in older adults. The other main problem of circadian rhythm disorder is called irregular sleep-wake rhythm, where people are unable to set a sleep pattern no matter how hard they try. The spectrum of association between pregnancy and sleep disturbances ranges from an increased incidence of insomnia, nocturnal awakenings, and parasomnias (especially restless legs syndrome) to snoring and excessive sleepiness [9].

1.1.2 Sleep change in normal pregnancy [10]

Pregnancy-related fatigue is highly related to sleep duration and quality. Progesterone is a hormone that is often associated with pregnancy-related fatigue [11]. Besides the influence of hormones, sleep changes during pregnancy are commonly caused by physical discomfort as the uterus gets bigger and the fetus grows, coupled with pregnancy-related weight gain and fluid accumulation in the body. Emotional factors can also play a role: pregnancy-related fatigue can come from the excitement and anticipation of having a baby, the fears of impending motherhood and the anxiety about labor and delivery.

Hormonal changes are steep during the first trimester (T1), slow down during the second trimester (T2), and then are steep again in the third trimester (T3). During the first trimester, rising progesterone levels not only make a woman feel fatigue, but they may also be partly to blame for the frequent need to urinate, which can also disrupt sleep and worsen sleepiness. The

second trimester of pregnancy is usually the least problematic because things are not changing quite as quickly. Leg cramps and heartburn may occur at night during the second trimester and keep women awake. As a woman's belly size increases and the fetus gets bigger and more active, the third trimester can generate severe sleep problems.

1.1.3 Sleep disorders occurring in pregnancy

Sleep disturbances are a recognized problem in pregnancy, to the extent that the American Sleep Disorders Association has proposed the existence of "pregnancy-associated sleep disorder"[12]. Researchers have found that not getting enough sleep during pregnancy could affect a woman in ways that go beyond feeling exhaustion, irritability, and poor concentration [13]. Due to hormonal and physical factors, changes in breathing physiology during pregnancy predispose women to sleep-breathing disorders [14] and any condition that causes maternal hypoxemia will affect sleep negatively. Snoring by the end of pregnancy is associated with hypertension, a clinical condition related to reduction of fetal growth and low birth weight [15]. When normal sleep is impossible, restrictions in daily functioning or excessive daytime sleepiness (EDS) may also occur.

In a previous study, three hundred 11-to-40-year-old pregnant women were interviewed in the outpatients' clinic to investigate sleep disorders occurring in pregnancy [16]. Their sample of pregnant women presented: 143 cases of insomnia; 113 cases of sleep breathing disorder; 54 cases of EDS; 113 cases of mild sleepiness; and 22 cases of specific awakenings. Insomnia prevalence was not different between pregnant women in T1 and T3 when compared to the pre-pregnancy (PG) state. In T2, however, there was an increase of 23% in insomnia complaints. Sleep breathing disorders did not differ between the pre-pregnancy period and all trimesters of pregnancy. Meanwhile, EDS was increased by 15% in T1, 55% in T2 and 14% in T3. Mild

sleepiness was not different in T1, increased by 33% in T2, and by 48% in T3. Specific Awakenings were very prevalent in T1, T2 and T3 compared to the PG state (T1=63%; T2 =80%; T3=84% - Fig 1). They concluded that sleep disorders were more frequent during pregnancy comparatively to PG state, mostly at the expenses of EDS and specific awakenings[16].

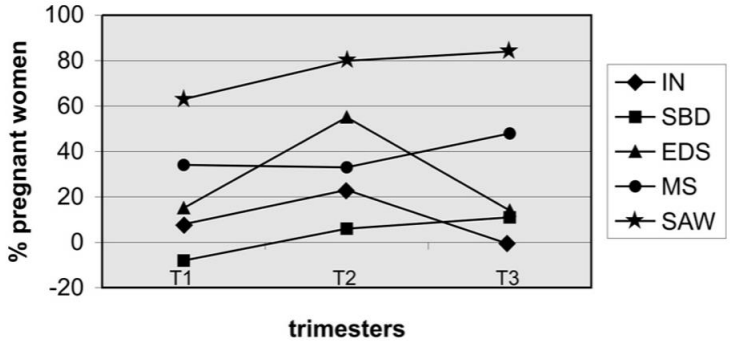

Figure 1. Differences among pregnant women with sleep disorders: insomnia (IN), sleep breathing disorders (SBD), excessive daytime sleepiness (EDS), mild sleepiness (MS) and specific awakenings (SAW) in the three trimesters of pregnancy (T1, T2 and T3).

1.2 Linear Regression and Estimation of Parameters

1.2.1 Linear regression model

Suppose we want to model the response Y in terms of two predictors, X_1, and X_2. One very general form for the model would be:

$$Y = f(X_1, X_2) + \varepsilon$$

where f is some unknown function and ε is the error. The typical assumption on this function with some more restricted form is linear as in:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \varepsilon$$

where $\beta_i, i = 0,1,2$ ... are unknown parameters with which β_0 is also called the intercept term. Though linear representation form is more restricted, each term could be transformed, which makes the linear model quite flexible. Thus, the problem is reduced to the estimation of

parameters rather than the infinite dimensional. The primary parameter to be considered is error ε. The Gauss-Markov assumptions concern the set of error random variables ε_i as below:

(1) They have mean zero: $\mathbf{E}[\varepsilon_i] = 0$;

(2) They are homoscedastic, that is all have the same finite variance: $\mathbf{Var}(\varepsilon_i) = \sigma^2 < \infty$;

(3) Distinct error terms are uncorrelated: $\mathbf{Cov}(\varepsilon_i, \varepsilon_j) = 0, \forall i \neq j.$

This regression model can be written as $y = X\beta + \varepsilon$, where $y \in R^n$ while $\beta \in R^p$, where p is the number of predictors or parameters. The problem is to find β so that Xβ is as close to Y as possible. Suppose we find a collection of $\hat{\beta}$ as the best β that make Xβ closest to Y. The $\hat{\beta}$ values are sometimes called the regression coefficients, and $\hat{y} = X\hat{\beta}$ are called predicted or fitted values. The difference between the actual response and the predicted response is denoted by $\hat{\varepsilon}$, which is called the residual. The usual way to find the best regression coefficients is by least squares estimation. This is a process of minimizing the residual sum squares (RSS) $\sum \varepsilon^2 = \varepsilon^T\varepsilon = (y - X\beta)^T(y - X\beta)$, and we confirm that the least squared estimate is the best possible estimates of β when the errors ε are uncorrected and have equal variance. The common choice of metric that measures how well the model fits the data is R2, also known as "coefficient of determination" or "percentage of variance explained":

$$R^2 = 1 - \frac{\sum(\hat{y_i} - y_i)^2}{\sum(y_i - \bar{y}_i)^2} = 1 - \frac{\text{RSS}}{\text{Total SS(Corrected for Mean)}}$$

Its range is [0, 1], where values closer to 1 indicate better fits. However, R^2 should not be used as the only metric to measure fitness, because sometimes different data share the same R^2 and this a nonlinear (quadratic) measure of model fit so 0.8 is not twice as good as 0.4, for instance.

1.2.2 Error and residual

In the context of discussion of errors term ε, we have assumed that the error is independent and identically distributed (i.i.d.) and furthermore we have also assumed that the errors are asymptotically normally distributed in order to carry out the usual statistical inference. However these assumptions are usually violated and alternatives must be considered, although the linear regression model is remarkably robust to minor violations of the Gauss-Markov assumptions. When the errors are dependent, generalized least squares (GLS) can be used. When the errors are independent, but not identically distributed, weighted least squares (WLS) can be used, which is a special case of GLS. When the errors are not normally distributed, robust regression can be used. There are some insights that show the benefits of applying robust estimators: 1. Robust estimators provide protection against long-tailed errors, but they cannot overcome problems with the choice of model and its variance structure. 2. Robust estimates supply $\hat{\beta}$ and possibly standard errors without the associated inferential methods. 3. Robust methods can be used in addition to least squares as a confirmatory method. This may cause the two estimates to diverge. In this case, the source of the difference should be investigated.

1.3 Model Selection

1.3.1 Goal

For all the simplest cases we are confronted with a choice of possible regression models for the data. Occam's Razor states that among several plausible explanations for a phenomenon, least assumptive is best. In regression analysis, this implies that the smallest model that fits the data adequately is best. Another consideration is that unnecessary predictors will introduce noise to the estimation of other quantities of interest. Therefore, variable selection is a means to an end

and not an end in itself. The aim is to construct a model that predicts or explains relationships in the data. Automatic variable selections are not guaranteed to be consistent with these goals. It is also important to note that biomedical data are highly correlated and typically contain significant measurement error. All of this suggests a cautious approach to model fitting.

1.3.2 Methods

Some models have a natural hierarchy. For example, in polynomial models, x^2 is a higher order term than x. When selecting variables, it is important to respect the hierarchy that lower order terms should not usually be removed from the model before higher order terms in the same variable.

Testing-Based Procedures: *Backward Elimination*, *Forward Selection* and *Stepwise Regression* are typical testing-based procedures for model selection.

Backward Elimination is the simplest of all variable selection procedures. It starts with all the predictors in the model and then removes the predictors with p-values higher than α_{crit}, where α_{crit} is called the "p-to-remove". Next we refit the model to remove the remaining least significant predictor provided its p-value is also greater than α_{crit}.

Forward Selection just reverses the backward method, which starts with no variables in the model and then adds predictors to the model one at a time by checking their p-values.

Stepwise Regression is a combination of the previous two. This addresses the situation where variables can be added or removed in each stage and repeated until there are no more variables to be added or removed.

However, in the case of biomedical data, none of these approaches are recommended. High levels of correlation impose path-dependent trajectories through model selection that typically

lead to non-optimal specifications. So the approach here is to let biological and medical theory guide the model selection and then to test alternatives with criterion-based procedures.

Criterion-Based Procedures: *AIC*, *BIC*, *Cp* are the typical metrics for Criterion-Based Procedures.

AIC: From the well known Kullback-Leibler information (or distance), we substitute in the MLE (maximum likehood estimate) of θ and rearrange to obtain:

$$\hat{I}(f,g) = \int f(x) log f(x) dx - \int f(x) log \mathrm{g}(x|\hat{\theta}) dx$$

(Akaike, 1974) showed that $E\hat{I}(f,g)$ can be estimated by

$$-logL(\hat{\theta}) + p + constant$$

Where p is the number of parameters in the model and the constant depends on the unknown true model. Akaike multipled this by two to obtain "an information criterion"(*AIC*):

$$AIC = -2L(\hat{\theta}) + 2p$$

For linear regression models, the -2 max log-likelihood, there is

$$-2L(\hat{\theta}) = nlog\left(\frac{RSS}{n}\right) + another\ constant$$

Since the constants are the same for a given dataset and assumed error distribution, they can be ignored in comparisons of regression models on the same data. *AIC* is used as a metric to minimize when choosing the model.

BIC: Most well-known among the alternatives is the Bayes information criterion (*BIC*), which is:

$$BIC = -2 \text{ max log-likelihood} + p\log n.$$

BIC penalizes larger models more heavily and so will tend to prefer smaller models in comparison to *AIC*.

Cp: The average mean square error of prediction might be a good criterion, as a good model should predict well, that is:

$$\frac{1}{\sigma^2}\sum_i E(\hat{y}_i - Ey_i)^2$$

Which can be estimated by Mallow's *Cp* statistic:

$$Cp = \frac{RSS_p}{\hat{\sigma}^2} + 2p - n$$

Where $\hat{\sigma}^2$ is from the model with all predictors and RSS_p indicates the *RSS* from a model with *p* parameters. For the full model *Cp* = *p* exactly. If a *p* predictor model fits. Then $E(RSS_p) = (n-p)\sigma^2$ and the $E(Cp) \approx p$. A model with a bad fit will have *Cp* much bigger than *p*. It is usual to plot *Cp* against *p*. We desire models with small *p* and *Cp* around or less than *p*. *Cp*, R_a^2, and *AIC* all trade-off fit in terms of RSS against complexity(*p*).

1.3.3 Summary

The aim of variable selections is to construct a model that predicts well or explains the relationships in the data. Automatic variable selections are not guaranteed to be consistent with these goals. If the models seem roughly comparable but lead to quite different conclusions, then it is clear that the data cannot answer the questions of interest unambiguously.

1.4 Categorical Predictors

Predictors that are qualitative in nature are sometimes described as *categorical* or called *factors,* and different categories of a factor variable are called levels. Sometimes an alternative coding of factor variables can be useful, especially for a categorical variable that has multiple levels. Let *B* be an $n \times k$ dummy variable matrix where $B_{ij} = 1$ if case *i* falls in class *j* and is zero otherwise. *B* might be used to from part of the model matrix. However, the row sums of *B*

are all one. The coding is determined by a *contrast matrix C* which has dimension $k \times (k-1)$. Contributions to the model matrix are then given by *BC*. Other columns of the model matrix might include a column of ones for the intercept and perhaps other predictors. Some classic coding methods include Treatment coding, Helmert coding, Polynomial coding, and Sum coding.

1.5 Models with Several Factors

Data with more than one factor could arise from observational studies or from designed experiments. If all possible combinations of the levels of the factors occur at least once, then it can be called full factorial design. Repeated observations for the same combination of factor levels are called replications. The usual procedure for modeling factors is first identifying if there are potential factor interactions, and then analysis of the model with ANOVA.

Suppose the dataset has factors $\alpha, \beta, \gamma, \ldots$ at levels $l_\alpha, l_\beta, l_\gamma, \ldots$. A full factorial experiment has at least one run for each combination of the levels. The number of combinations is $l_\alpha l_\beta l_\gamma \ldots$, which could easily be very large. For this reason, full factorials are rarely executed for more than three or four factors. Though, there are some advantages to factorial designs. If no interactions are present, we get several one-way experiments for the price of one. Comparing this with doing a sequence of one-way experiments, it is sometimes better to use replication for investigating another factor instead. The analysis of full factorial experiments is an extension of that used for the two-way ANOVA. Typically, there is no replication due to cost concerns so it is necessary to assume that some higher order interactions are zero in order to free up degrees of freedom for testing the lower order effects.

Chapter 2: Materials and Methods

2.1 Purpose

Several aims were established for the current study. First, an overview of the whole dataset will be constructed. Second, a full predictable linear regression with the most suitable predictors will be investigated. In this part, analysis for different periods of pregnancy will be discussed separately, since different factors are expected to predominate during different periods of pregnancy. Midpoint sleep time, melatonin peak value, and cortisol peak value are considered as response variables here. A discussion of factors for the models will be given at the end.

2.2 Dataset

2.2.1 Overview

Participants were women who planned to be pregnant (N=291), with random selection before their pregnancy from Barnes Jewish Hospital and Medical Center. All human studies were done in accordance with protocols approved by Washington University Institutional Review Board (IRB). A questionnaire containing 713 questions were collected several times during baseline, the first trimester, the second trimester, and the third trimester. For those who have not been pregnant or who have not delivered, records only exist on their baseline and early pregnancy. By the time this study was done, there were 212 participants who received the questionnaire before their pregnancy, which we treated as a baseline. Seventy-three of those participants finished their questionnaire in the first trimester. Data for the second trimester and the third trimester were collected from 69 and 60 of them, respectively.

Data was collected, stored and manipulated in RedCap by Washington University, Center for Biomedical Informatics. Questions were split into different parts, including background, hormone and behavior. The background questions related to race, income, education, etc. Hormone reports included testing results of melatonin and cortisol, which are the two well-known essential hormones affecting sleep. These tests were done several times during baseline and each trimester. A calculation of the peak value of melatonin and cortisol for each person during each period was done to help construct the model

Behavioral assays part included a large volume of information, which was separated as sleep related factors (SRF), job related factors (JRF), after-work related factors (AWRF), medication and nutrition related factors (MRF), and others. SRF had questions like: “Do you wake up by using or not using an alarm?” (*alarmclock*); “How many times can you not get to sleep within 30 minute a week?” (*slp30*); “How often do you take medicine to sleep?” (*slpmed*); and “Have you ever been to a sleep clinic?” (*sleepclinic*). JRF had questions like: “Do you have a paid job?” (*paidjob*); “In comparison with other woman of your age, do you think your work is physically lighter or heavier” (*workload_weight*); “In the last 3 months, were you a shift or night worker?” (*nightshift*); “How many work shift changes have you had?” (*shiftchanges*); “When you are working at your current occupation, how often do you sweat from exertion?” (*job_sweat*) and “How many minutes a day do you usually walk and/or bicycle to and from work, school, or errands?” (*workwalk*). AWRF had questions like: “How often did you play sports or exercise?” (*playsports*); “How many hours do you do your favorite sport or exercise every week?” (*activity_hours*) and “After work, are you physically tired?” (*after_work*); “Are you caring for a child or children between 2 and 5 years of age?” (*caretoddler*); and “are you caring for a child or children under 2 years of age?” (*under2care*). Within MRF group, only “whether use vitamins or

not (*vitamins*)" was chosen to use in this study, because it was found that disturbed sleep maintenance was associated with multi-/multiple vitamin use [17]. Other MRF were found either to be too complexed or to have low relationship with sleep, so they were not discussed in this study.

2.2.2 Categorical Variables

Categorical Variables	Descriptions	Categories		%
alarmclock	Use alarmclock or not?	·	Missing (NA)	5.84
		0	No	18.25
		1	Yes	75.91
shiftchange	How many work shift changes in the past month?	.	Missing (NA)	9.25
		0	No	73.72
		1	1	8.76
		2	2	4.62
		3	3 or more	3.65
nightchange	Being a night/shift worker?	·	Missing (NA)	2.43
		0	No	74.70
		1	Yes	22.87
slpmed	Use sleep medication or not?	.	Missing (NA)	1.70
		0	No	84.18
		1	one or two times a week	9.74
		2	three or more times a week	4.38
slp30	How many times not get to sleep within 30 minute a week?	.	Missing (NA)	1.70
		0	Less than 1 time a month	36.50
		1	one or two times a week	46.72
		2	three or more times a week	15.08
race	What race category?	.	Missing (NA)	5.35
		1	White	58.88
		2	Black	27.25
		3	Others	8.52
workload_weight	Work is physically light or heavy?	·	Missing (NA)	3.89
		1	Light	24.57
		2	Medium	48.91
		3	Heavy	22.63
after_work	After work, physically	·	Missing (NA)	3.65

	tired or not?	0	Never tired	4.14
		1	Sometimes tired	55.71
		2	Often tired	36.50
job_sweat	How often sweat from exertion?	.	Missing (NA)	3.89
		0	Never sweat	46.72
		1	Sometimes sweat	44.77
		2	Often sweat	4.62
playsports	How often play sports or exercise?	.	Missing (NA)	1.70
		0	Never	19.96
		1	Rarely	22.38
		2	Often	55.96
vitamins	Use vitamins or not?	.	Missing (NA)	1.70
		0	Do not use	39.90
		1	Use	58.40
sleepclinic	Ever been to a sleep clinic?	.	Missing (NA)	2.19
		0	No	92.70
		1	Yes	5.11
highbp	Have high blood pressure?	.	Missing (NA)	3.65
		0	No	88.56
		1	Yes	7.79
prior_pregnancy	Ever been pregnant?	.	Missing (NA)	1.70
		0	No	41.85
		1	Yes	56.45
under2care	Caring for a child or children under 2 years of age?	.	Missing (NA)	1.95
		0	No	75.67
		1	Yes	22.38
caretoddler	Caring for a child or children between 2 and 5 years of age?	.	Missing (NA)	1.70
		0	No	74.45
		1	Yes	23.85
paidjob	Have a paid job or not?	.	Missing (NA)	1.95
		0	No	11.92
		1	Yes	86.13

Table 1: Categorical variables

2.2.3 Numerical Variables

Variable	Description	Range		Mean	Median	Missing	Units
		Lower	upper				
midpoint	Midpoint sleep time	0.25	23.75	14.94	14.87	7.66%	time (set 12:00pm as 0)
melatonin	Melatonin value	1.198	2327.294	41.232	22.653	4.87%	pg/ml
cortisol	Cortisol value	0.423	392.2	5.597	4.212	7.79%	ng/ml

Table 2: Numerical variables

2.2 Software

The R statistical environment software was used for variable transformation, data cleaning, correlation analysis, model specification, influence testing and the creation of graphs.

The specific R packages used here are listed below:

"arm"; "lme4", "car", "corrgram", "mice".

2.4 Procedures

Initially, the dataset was explored as a whole, where a complete model including all the variables was developed. Formatting time variables to 24-hour standard format was the first thing to be considered. In order to discuss a linear regression for midpoint sleep time, it was necessary to treat 12 pm as zero, using the function *trans2time* (Appendix 1). Participants had their melatonin and cortisol levels measured at different time during the day, depending on when they came to the hospital. Peak values were calculated according to the function *find_maxmaxval* (Appendix 2). Midpoint sleep time was calculated by using mean of bed time and wake-up time.

Some variables, including *race*, *workload_weight* and *afterwork*, were cleaned up by combining some levels together. For example, the original *race* had 11 levels, which were: 1.White, 2. Black or African America, 3. American Indian or Alaskan Native, 4. Asian Indian, 5. Chinese, 6. Japanese, 7. Korean, 8. Vietnamese, 9. Some other race, 10. Don't know and 11.

Refused. There were no participants in level 3, 6, 7, 8, 10, and small numbers of participants in level group 4, 5, 9, and 11, which comprises 8.52% of the dataset. Given these results, we reset levels to 1.White, 2. Black or African America, and 3. All others. Other variables were combined in the same way.

Then, two variables -- *period* and *weekday* were added to the dataset. Data from non-pregnancy, the 1st trimester, the 2nd trimester, and the 3rd trimester were labeled as level 0, 1, 2, 3 respectively in *period*. *Weekday* was used to clarify whether data came from workday (level 1) or free day (level 0).

After all factors were set, we performed ANOVA and correlation analyses to find all significantly effective factors. Based on these results and our knowledge of the biologically important effects, linear models were built for different parameters and different periods of pregnancy. All models were considered carefully by looking at their residuals and leverages, and then good ones were selected at the end. In order to look at the models differences at different periods, a multilevel model was further studied.

Chapter 3: Results

3.1 Hormone and Midpoint Sleep Summary

Based on the information provided in section 1.1, the midpoint sleeping time and hormone levels were treated as the most important for this study. The plotting procedure was separated by weekday (Figure 2) and weekend (Figure 2). It is obvious that the peak of melatonin occurs around the midpoint sleeping time and the peak of cortisol appears around the wakeup point, as expected. There is less variance shown in weekends than weekdays, because these two hormones and sleeping are associated with labor work and job related stress, which could vary a lot within the female population. Importantly however, we also demonstrated that the second trimester has the highest melatonin peak value among all periods, and this value shifted more to the midpoint sleeping time, especially at weekend (Figure 2.E and Figure 3.E).

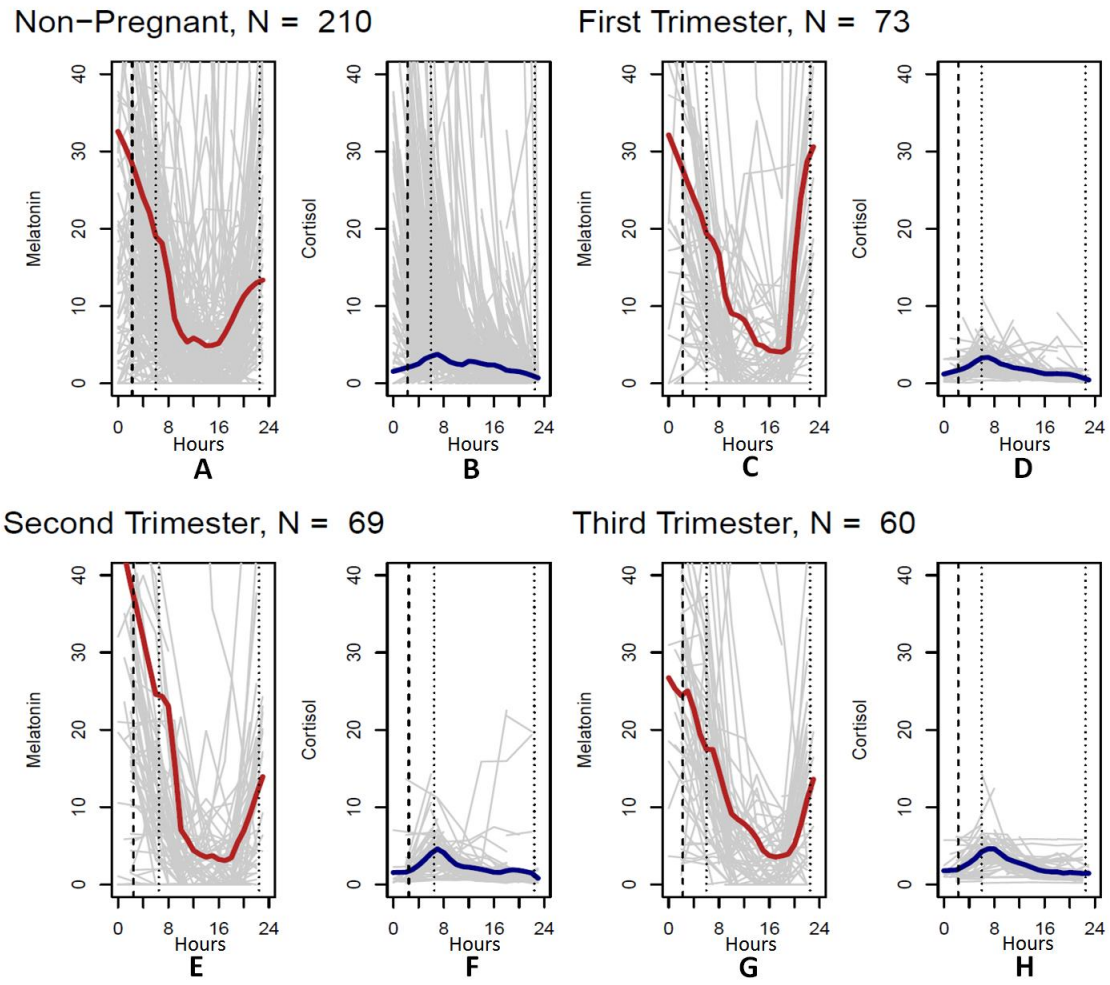

Figure 2: Melatonin and Cortisol levels during weekdays for non-pregnant and three pregnant periods. Dotted lines indicatesleep start/stop, and dashed lines indicate midpoint.

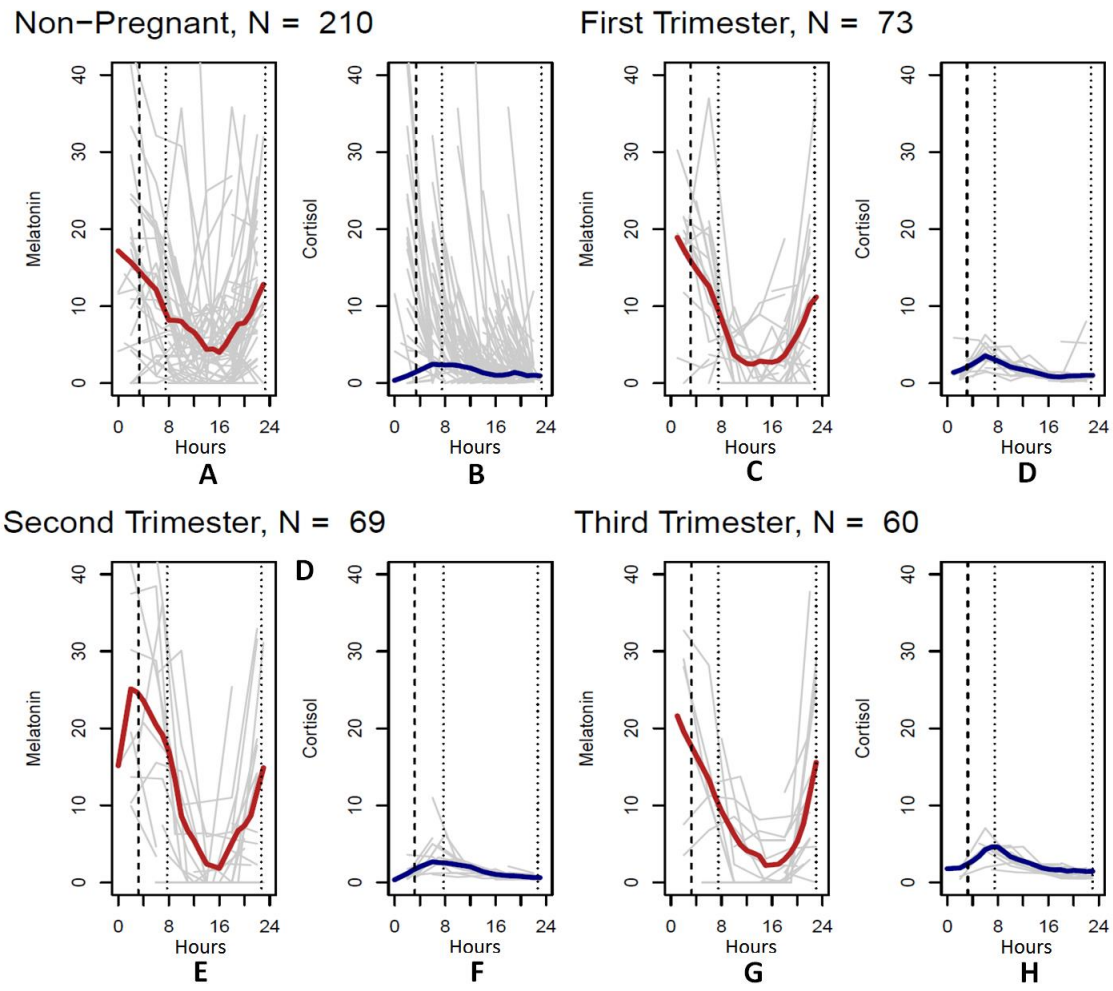

Figure 3: Melatonin and Cortisol levels during weekends for non-pregnant and three pregnant periods. Dotted lines for sleep start/stop, and dashed line for their midpoint.

3.2 ANOVA Analysis for All Factors

In order to investigate changes in hormone and midpoint sleep time during weekdays and free days in different periods, ANOVA analysis on all factors was considered before running our models.

3.2.1 ANOVA analysis for non-pregnancy

ANOVA analysis table of midpoint sleep time showed effective factors of *nightshift*, *slp30* and *job_sweat* during weekdays (Table3 A), and *slp30* during weekend (Table3D). There was no

factor found to have significant effect on melatonin levels during either weekday (Table3 B) or weekend (Table3 E). Even though no factor was found to have an effect on cortisol level during weekday (Table3 C), *shiftchange* had a significant effect on cortisol level on weekend with a p-value equals to 0.04733 (Table3 F).

Weekday Midpoint Sleep ANOVA Table

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
alarmclock	1	9.33	9.325	2.1211	0.148269
shiftchange	3	5.07	1.691	0.3847	0.764257
nightshift	1	104.40	104.399	23.7465	3.911e-06
slp30	2	46.78	23.392	5.3206	0.006295
slpmed	2	13.74	6.868	1.5622	0.214504
under2care	1	3.60	3.596	0.8180	0.367849
caretoddler	1	3.48	3.482	0.7921	0.375507
workload_weight	2	13.54	6.772	1.5403	0.219130
after_work	2	5.71	2.857	0.6498	0.524252
job_sweat	2	42.21	21.105	4.8006	0.010116
playsports	2	5.40	2.700	0.6140	0.543098
vitamins	1	0.00	0.000	0.0000	0.994390
sleepclinic	1	0.47	0.466	0.1059	0.745465
highbp	1	9.15	9.152	2.0817	0.152051
prior_pregnancy	1	0.85	0.848	0.1929	0.661386
paidjob	1	0.19	0.191	0.0434	0.835346
race	2	11.44	5.722	1.3014	0.276492
Residuals	105	461.62	4.396		

A

Weekday Melatonin ANOVA Table

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
alarmclock	1	124	123.57	0.2286	0.63357
shiftchange	3	999	333.01	0.6160	0.60613
nightshift	1	34	33.85	0.0626	0.80291
slp30	2	371	185.43	0.3430	0.71042
slpmed	2	922	461.16	0.8531	0.42903
under2care	1	1023	1022.85	1.8921	0.17189
caretoddler	1	1671	1671.26	3.0916	0.08161
workload_weight	2	279	139.60	0.2582	0.77291
after_work	2	102	51.22	0.0947	0.90968
job_sweat	2	444	221.77	0.4102	0.66455
playsports	2	50	24.81	0.0459	0.95517
vitamins	1	91	91.35	0.1690	0.68185
sleepclinic	1	206	205.73	0.3806	0.53864
highbp	1	412	411.58	0.7613	0.38490
prior_pregnancy	1	688	687.59	1.2719	0.26197
paidjob	1	416	415.64	0.7689	0.38257
race	2	314	156.91	0.2903	0.74867
Residuals	105	56762	540.59		

B

Weekday Cortisol ANOVA Table

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
alarmclock	1	13.06	13.0553	2.0659	0.15360
shiftchange	3	43.82	14.6052	2.3111	0.08046
nightshift	1	1.31	1.3125	0.2077	0.64952
slp30	2	0.88	0.4422	0.0700	0.93247
slpmed	2	11.82	5.9102	0.9352	0.39574
under2care	1	4.64	4.6422	0.7346	0.39335
caretoddler	1	2.50	2.5012	0.3958	0.53064
workload_weight	2	0.88	0.4422	0.0700	0.93246
after_work	2	0.52	0.2576	0.0408	0.96007
job_sweat	2	22.21	11.1065	1.7575	0.17751
playsports	2	9.16	4.5817	0.7250	0.48673
vitamins	1	3.33	3.3335	0.5275	0.46928
sleepclinic	1	3.61	3.6091	0.5711	0.45152
highbp	1	0.19	0.1894	0.0300	0.86289
prior_pregnancy	1	7.90	7.8950	1.2493	0.26624
paidjob	1	5.56	5.5559	0.8792	0.35058
race	2	11.90	5.9511	0.9417	0.39323
Residuals	105	663.55	6.3195		

C

Weekend Midpoint Sleep ANOVA Table

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
alarmclock	1	0.521	0.5212	0.3123	0.5775458
shiftchange	3	6.861	2.2871	1.3703	0.2562575
nightshift	1	0.651	0.6507	0.3899	0.5337937
slp30	2	26.745	13.3724	8.0122	0.0005923
slpmed	2	2.556	1.2779	0.7657	0.4677254
under2care	1	0.023	0.0229	0.0137	0.9069181
caretoddler	1	0.922	0.9219	0.5524	0.4590975
workload_weight	2	1.958	0.9792	0.5867	0.5580596
after_work	2	6.839	3.4195	2.0488	0.1342664
job_sweat	2	3.985	1.9927	1.1939	0.3073084
playsports	2	1.640	0.8201	0.4914	0.6132495
vitamins	1	4.171	4.1707	2.4989	0.1170825
sleepclinic	1	0.022	0.0215	0.0129	0.9098389
highbp	1	0.004	0.0044	0.0026	0.9591263
prior_pregnancy	1	0.486	0.4859	0.2911	0.5907116
paidjob	1	0.001	0.0007	0.0004	0.9837856
race	2	2.103	1.0516	0.6301	0.5346392
Residuals	100	166.901	1.6690		

D

Weekend Melatonin ANOVA Table

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
alarmclock	1	127	127.23	0.2257	0.63573
shiftchange	3	769	256.43	0.4550	0.71434
nightshift	1	1	1.18	0.0021	0.96360
slp30	2	289	144.53	0.2564	0.77430
slpmed	2	867	433.54	0.7692	0.46608
under2care	1	787	786.93	1.3963	0.24015
caretoddler	1	1933	1933.38	3.4305	0.06695
workload_weight	2	417	208.72	0.3703	0.69144
after_work	2	3	1.69	0.0030	0.99700
job_sweat	2	370	185.17	0.3286	0.72074
playsports	2	125	62.41	0.1107	0.89528
vitamins	1	141	141.48	0.2510	0.61744
sleepclinic	1	159	158.96	0.2820	0.59654
highbp	1	411	411.19	0.7296	0.39505
prior_pregnancy	1	743	743.39	1.3190	0.25351
paidjob	1	637	636.81	1.1299	0.29035
race	2	383	191.73	0.3402	0.71245
Residuals	100	56359	563.59		

E

Weekend Cortisol ANOVA Table

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
alarmclock	1	12.91	12.9114	2.1187	0.14864
shiftchange	3	50.08	16.6942	2.7395	0.04733
nightshift	1	0.64	0.6447	0.1058	0.74567
slp30	2	1.55	0.7744	0.1271	0.88081
slpmed	2	28.54	14.2714	2.3419	0.10140
under2care	1	2.40	2.4029	0.3943	0.53147
caretoddler	1	4.04	4.0447	0.6637	0.41718
workload_weight	2	2.00	1.0019	0.1644	0.84862
after_work	2	0.61	0.3034	0.0498	0.95146
job_sweat	2	24.67	12.3326	2.0237	0.13754
playsports	2	4.78	2.3921	0.3925	0.67638
vitamins	1	1.85	1.8499	0.3036	0.58289
sleepclinic	1	3.27	3.2662	0.5360	0.46582
highbp	1	0.00	0.0001	0.0000	0.99654
prior_pregnancy	1	7.64	7.6363	1.2531	0.26564
paidjob	1	4.44	4.4434	0.7291	0.39520
race	2	23.29	11.6447	1.9109	0.15332
Residuals	100	609.39	6.0939		

F

Table 3: ANOVA tables for non-pregnancy period: A for weekday midpoint sleep time; B for weekday melatonin level; C for weekday cortisol level; D for weekend midpoint sleep time; E for weekend melatonin level; and F for weekend cortisol level.

3.2.2 ANOVA analysis for the first trimester

From the first trimester, pregnant women started having physical changes corresponding with sleeping problems. At the first trimester, *slp30* and *caretoddler* had significant effects on the midpoint sleep time during weekdays (Table4 A), but lost their effect on weekends (Table4 D). Surprisingly, *prior_pregnacy* was found to have a marked influence on the midpoint sleep on weekends (Table4 D). It was very interesting that the melatonin level had very similar effective factors on weekday and weekend, which were *alarmclock*, *slpmed*, and *paidjob*, which all had p-value less than 0.001(Table4 B&E). The *prior_pregnancy* had a significant effect on the cortisol levels on weekday (Table4 C). However, during weekend, nothing was found to have significant effect on the cortisol levels (Table4 F).

Weekday Midpoint Sleep ANOVA Table

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
alarmclock	1	1.162	1.1620	0.6745	0.418418
shiftchange	3	2.274	0.7582	0.4401	0.726113
nightshift	1	6.934	6.9343	4.0253	0.054570
slp30	2	37.702	18.8508	10.9426	0.000308
slpmed	2	4.487	2.2434	1.3023	0.287879
under2care	1	1.220	1.2198	0.7081	0.407215
caretoddler	1	8.471	8.4712	4.9174	0.034877
workload_weight	2	3.743	1.8714	1.0863	0.351262
after_work	2	0.413	0.2065	0.1198	0.887507
job_sweat	2	0.895	0.4474	0.2597	0.773095
playsports	2	7.864	3.9320	2.2824	0.120703
vitamins	1	0.005	0.0048	0.0028	0.958251
sleepclinic	1	0.420	0.4203	0.2440	0.625218
highbp	1	6.264	6.2638	3.6360	0.066850
prior_pregnancy	1	6.930	6.9302	4.0229	0.054638
paidjob	1	0.477	0.4765	0.2766	0.603063
race	2	6.483	3.2416	1.8817	0.171098
Residuals	28	48.236	1.7227		

A

Weekday Melatonin ANOVA Table

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
alarmclock	1	162198	162198	16.7257	0.0003302
shiftchange	3	11957	3986	0.4110	0.7463426
nightshift	1	161	161	0.0166	0.8984686
slp30	2	21880	10940	1.1281	0.3379024
slpmed	2	227618	113809	11.7359	0.0001987
under2care	1	2232	2232	0.2301	0.6351460
caretoddler	1	21	21	0.0022	0.9633102
workload_weight	2	4030	2015	0.2078	0.8136414
after_work	2	15977	7988	0.8238	0.4491306
job_sweat	2	16217	8108	0.8361	0.4439224
playsports	2	12464	6232	0.6426	0.5334891
vitamins	1	4259	4259	0.4392	0.5129515
sleepclinic	1	676	676	0.0697	0.7937622
highbp	1	85	85	0.0088	0.9259438
prior_pregnancy	1	15078	15078	1.5549	0.2227470
paidjob	1	242200	242200	24.9754	2.792e-05
race	2	14871	7436	0.7668	0.4740231
Residuals	28	271530	9698		

B

Weekday Cortisol ANOVA Table

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
alarmclock	1	1.135	1.1355	0.3668	0.54962
shiftchange	3	10.072	3.3575	1.0847	0.37170
nightshift	1	0.362	0.3617	0.1169	0.73502
slp30	2	15.143	7.5713	2.4460	0.10494
slpmed	2	5.034	2.5172	0.8132	0.45363
under2care	1	0.001	0.0010	0.0003	0.98603
caretoddler	1	0.991	0.9905	0.3200	0.57611
workload_weight	2	0.951	0.4753	0.1536	0.85837
after_work	2	1.983	0.9913	0.3202	0.72859
job_sweat	2	5.742	2.8709	0.9275	0.40736
playsports	2	0.516	0.2581	0.0834	0.92022
vitamins	1	12.531	12.5310	4.0483	0.05393
sleepclinic	1	0.855	0.8553	0.2763	0.60326
highbp	1	1.357	1.3571	0.4384	0.51330
prior_pregnancy	1	13.814	13.8139	4.4628	0.04369
paidjob	1	0.195	0.1952	0.0630	0.80358
race	2	8.475	4.2373	1.3689	0.27089
Residuals	28	86.670	3.0954		

C

Weekend Midpoint Sleep ANOVA Table

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
alarmclock	1	4.254	4.254	0.4196	0.52260
shiftchange	3	2.930	0.977	0.0963	0.96139
nightshift	1	1.201	1.201	0.1185	0.73337
slp30	2	12.880	6.440	0.6353	0.53751
slpmed	2	6.257	3.128	0.3086	0.73701
under2care	1	2.449	2.449	0.2416	0.62705
caretoddler	1	2.664	2.664	0.2628	0.61240
workload_weight	2	36.320	18.160	1.7915	0.18596
after_work	2	18.983	9.492	0.9363	0.40442
job_sweat	2	2.890	1.445	0.1425	0.86779
playsports	2	0.016	0.008	0.0008	0.99921
vitamins	1	5.379	5.379	0.5306	0.47261
sleepclinic	1	0.090	0.090	0.0088	0.92580
highbp	1	9.806	9.806	0.9673	0.33408
prior_pregnancy	1	49.331	49.331	4.8666	0.03608
paidjob	1	1.392	1.392	0.1374	0.71382
race	2	1.910	0.955	0.0942	0.91040
Residuals	27	273.692	10.137		

D

Weekend Melatonin ANOVA Table

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
alarmclock	1	162448	162448	16.2724	0.0004046
shiftchange	3	11779	3926	0.3933	0.7587962
nightshift	1	31	31	0.0031	0.9560341
slp30	2	21618	10809	1.0827	0.3529225
slpmed	2	227709	113855	11.4048	0.0002569
under2care	1	2023	2023	0.2026	0.6561879
caretoddler	1	2	2	0.0002	0.9900753
workload_weight	2	3078	1539	0.1542	0.8578877
after_work	2	15344	7672	0.7685	0.4735892
job_sweat	2	23620	11810	1.1830	0.3217409
playsports	2	13039	6519	0.6530	0.5284844
vitamins	1	6198	6198	0.6209	0.4375864
sleepclinic	1	376	376	0.0376	0.8476140
highbp	1	151	151	0.0151	0.9030063
prior_pregnancy	1	18316	18316	1.8347	0.1867974
paidjob	1	236027	236027	23.6426	4.409e-05
race	2	12321	6161	0.6171	0.5469446
Residuals	27	269543	9983		

E

Weekend Cortisol ANOVA Table

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
alarmclock	1	0.418	0.4179	0.1121	0.74022
shiftchange	3	8.225	2.7417	0.7352	0.53955
nightshift	1	0.041	0.0414	0.0111	0.91678
slp30	2	13.767	6.8836	1.8459	0.17596
slpmed	2	4.580	2.2902	0.6141	0.54801
under2care	1	0.602	0.6023	0.1615	0.69071
caretoddler	1	1.655	1.6549	0.4438	0.51058
workload_weight	2	2.330	1.1649	0.3124	0.73414
after_work	2	5.918	2.9591	0.7935	0.46184
job_sweat	2	21.303	10.6514	2.8562	0.07375
playsports	2	2.409	1.2043	0.3229	0.72658
vitamins	1	5.637	5.6370	1.5116	0.22878
sleepclinic	1	0.120	0.1204	0.0323	0.85862
highbp	1	1.690	1.6902	0.4532	0.50613
prior_pregnancy	1	8.041	8.0412	2.1563	0.15275
paidjob	1	0.006	0.0058	0.0016	0.96872
Residuals	29	108.147	3.7292		

F

Table 4: ANOVA tables for the first trimester: A for weekday midpoint sleep time; B for weekday melatonin level; C for weekday cortisol level; D for weekend midpoint sleep time; E for weekend melatonin level; and F for weekend cortisol level.

3.2.3 ANOVA analysis for the second trimester

The factors which affected midpoint sleep time and melatonin level during the second trimester were very similar to the first trimester. This is expected, because women during the second trimester had very similar sleeping problems compared to the first trimester. But, *race* was an important factor that significantly affected cortisol level. At the same time, *shiftchange* and *slp30* had significant effects on weekend cortisol level.

Weekday Midpoint Sleep ANOVA Table

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
alarmclock	1	1.079	1.0786	0.5207	0.477528
shiftchange	3	0.670	0.2233	0.1078	0.954736
nightshift	1	5.118	5.1182	2.4706	0.129086
slp30	2	31.316	15.6579	7.5581	0.002846
slpmed	2	3.419	1.7097	0.8253	0.450168
under2care	1	0.809	0.8089	0.3905	0.537950
caretoddler	1	10.203	10.2029	4.9250	0.036176
workload_weight	2	2.306	1.1531	0.5566	0.580365
after_work	2	1.725	0.8624	0.4163	0.664165
job_sweat	2	0.694	0.3470	0.1675	0.846775
playsports	2	13.223	6.6115	3.1914	0.059019
vitamins	1	1.775	1.7749	0.8567	0.363868
sleepclinic	1	0.224	0.2236	0.1079	0.745386
highbp	1	0.851	0.8505	0.4106	0.527755
prior_pregnancy	1	7.901	7.9015	3.8141	0.062580
paidjob	1	0.076	0.0757	0.0365	0.849997
race	2	11.972	5.9861	2.8895	0.075093
Residuals	24	49.720	2.0717		

A

Weekday Melatonin ANOVA Table

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
alarmclock	1	171718	171718	12.2766	0.0018251
shiftchange	3	19634	6545	0.4679	0.7074105
nightshift	1	1508	1508	0.1078	0.7455297
slp30	2	43636	21818	1.5598	0.2307406
slpmed	2	277581	138790	9.9226	0.0007236
under2care	1	2063	2063	0.1475	0.7043146
caretoddler	1	5345	5345	0.3822	0.5422704
workload_weight	2	38001	19000	1.3584	0.2761349
after_work	2	19061	9530	0.6814	0.5154514
job_sweat	2	30776	15388	1.1001	0.3490372
playsports	2	45310	22655	1.6197	0.2188639
vitamins	1	3186	3186	0.2277	0.6375159
sleepclinic	1	11147	11147	0.7969	0.3808788
highbp	1	1332	1332	0.0952	0.7602716
prior_pregnancy	1	48318	48318	3.4544	0.0753898
paidjob	1	276984	276984	19.8025	0.0001682
race	2	26077	13039	0.9322	0.4074915
Residuals	24	335696	13987		

B

Weekday Cortisol ANOVA Table

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
alarmclock	1	8.334	8.334	0.9351	0.343175
shiftchange	3	18.928	6.309	0.7079	0.556695
nightshift	1	29.534	29.534	3.3140	0.081187
slp30	2	50.540	25.270	2.8355	0.078444
slpmed	2	4.914	2.457	0.2757	0.761409
under2care	1	6.353	6.353	0.7128	0.406837
caretoddler	1	24.719	24.719	2.7737	0.108828
workload_weight	2	14.524	7.262	0.8149	0.454576
after_work	2	19.836	9.918	1.1129	0.344980
job_sweat	2	12.607	6.304	0.7073	0.502953
playsports	2	2.416	1.208	0.1355	0.873925
vitamins	1	3.460	3.460	0.3882	0.539102
sleepclinic	1	4.714	4.714	0.5289	0.474099
highbp	1	1.053	1.053	0.1182	0.734028
prior_pregnancy	1	3.329	3.329	0.3736	0.546816
paidjob	1	0.116	0.116	0.0130	0.910177
race	2	137.492	68.746	7.7138	0.002588
Residuals	24	213.891	8.912		

C

Weekend Midpoint Sleep ANOVA Table

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
alarmclock	1	6.102	6.1019	0.5289	0.4738
shiftchange	3	7.936	2.6454	0.2293	0.8751
nightshift	1	1.886	1.8864	0.1635	0.6894
slp30	2	3.923	1.9616	0.1700	0.8446
slpmed	2	2.143	1.0717	0.0929	0.9116
under2care	1	3.430	3.4299	0.2973	0.5904
caretoddler	1	4.128	4.1277	0.3578	0.5551
workload_weight	2	40.221	20.1104	1.7431	0.1956
after_work	2	7.289	3.6446	0.3159	0.7320
job_sweat	2	2.712	1.3562	0.1176	0.8896
playsports	2	3.147	1.5734	0.1364	0.8732
vitamins	1	0.022	0.0225	0.0019	0.9651
sleepclinic	1	1.022	1.0223	0.0886	0.7684
highbp	1	15.920	15.9200	1.3799	0.2512
prior_pregnancy	1	25.421	25.4212	2.2034	0.1502
paidjob	1	0.000	0.0000	0.0000	0.9989
race	2	3.148	1.5740	0.1364	0.8731
Residuals	25	288.435	11.5374		

D

Weekend Melatonin ANOVA Table

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
alarmclock	1	151426	151426	10.9558	0.0028345
shiftchange	3	16175	5392	0.3901	0.7611436
nightshift	1	695	695	0.0503	0.8243485
slp30	2	30475	15237	1.1024	0.3476670
slpmed	2	284859	142430	10.3049	0.0005445
under2care	1	56	56	0.0040	0.9499244
caretoddler	1	2649	2649	0.1917	0.6652856
workload_weight	2	33296	16648	1.2045	0.3166549
after_work	2	25237	12619	0.9130	0.4142985
job_sweat	2	33500	16750	1.2119	0.3145361
playsports	2	35100	17550	1.2697	0.2984021
vitamins	1	3062	3062	0.2216	0.6419259
sleepclinic	1	10753	10753	0.7780	0.3861596
highbp	1	147	147	0.0106	0.9186595
prior_pregnancy	1	19322	19322	1.3979	0.2481936
paidjob	1	353326	353326	25.5634	3.224e-05
race	2	10435	5217	0.3775	0.6894324
Residuals	25	345538	13822		

E

Weekend Cortisol ANOVA Table

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
alarmclock	1	18.958	18.958	1.7515	0.197665
shiftchange	3	115.471	38.490	3.5561	0.028578
nightshift	1	19.087	19.087	1.7635	0.196190
slp30	2	76.248	38.124	3.5223	0.044907
slpmed	2	13.911	6.955	0.6426	0.534390
under2care	1	2.397	2.397	0.2215	0.641986
caretoddler	1	18.936	18.936	1.7495	0.197907
workload_weight	2	21.605	10.802	0.9980	0.382821
after_work	2	10.992	5.496	0.5078	0.607920
job_sweat	2	13.601	6.800	0.6283	0.541723
playsports	2	2.809	1.404	0.1298	0.878900
vitamins	1	20.348	20.348	1.8799	0.182526
sleepclinic	1	0.508	0.508	0.0469	0.830288
highbp	1	7.456	7.456	0.6889	0.414403
prior_pregnancy	1	0.823	0.823	0.0760	0.784992
paidjob	1	5.215	5.215	0.4818	0.493987
race	2	134.057	67.028	6.1927	0.006538
Residuals	25	270.592	10.824		

F

Table 5: ANOVA tables for the second trimester: A for weekday midpoint sleep time; B for weekday melatonin level; C for weekday cortisol level; D for weekend midpoint sleep time; E for weekend melatonin level; and F for weekend cortisol level.

3.2.4 ANOVA analysis for the third trimester

Upon reaching the third trimester, pregnant women started having more sleep problems, which were effected by different factors as shown in Table 6. Midpoint sleep seems to have many effectors, so a good model needs to be built considering all these factors and some interactions. *vitamins* was an important factor that significantly affected melatonin level (Table6 B&E).*alarmclock* and *caretoddler* had significant effect on weekend melatonin level (Table6 E). During this period, *nightshift* had significant effects on cortisol level in both weekday and weekend (Table6 C&F). *Job_sweat* was also found to affect the cortisol level on weekday as well (Table6 C).

Weekday Midpoint Sleep ANOVA Table

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
alarmclock	1	1.5535	1.5535	10.2906	0.0075214
shiftchange	3	1.3668	0.4556	3.0178	0.0717518
nightshift	1	1.1741	1.1741	7.7773	0.0163816
slp30	2	3.2903	1.6452	10.8976	0.0020043
slpmed	2	0.6358	0.3179	2.1057	0.1645048
under2care	1	0.6208	0.6208	4.1120	0.0653802
caretoddler	1	0.2353	0.2353	1.5583	0.2357148
workload_weight	2	3.7047	1.8523	12.2698	0.0012546
after_work	2	1.2693	0.6347	4.2039	0.0413332
job_sweat	1	4.2748	4.2748	28.3164	0.0001819
playsports	2	0.4216	0.2108	1.3963	0.2849851
vitamins	1	1.0331	1.0331	6.8431	0.0225533
sleepclinic	1	0.1467	0.1467	0.9719	0.3436776
highbp	1	0.2057	0.2057	1.3624	0.2657887
prior_pregnancy	1	0.5105	0.5105	3.3814	0.0908003
paidjob	1	0.2324	0.2324	1.5397	0.2383783
race	2	0.6497	0.3248	2.1517	0.1590036
Residuals	12	1.8116	0.1510		

A

Weekday Melatonin ANOVA Table

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
alarmclock	1	742.50	742.50	3.2398	0.09704
shiftchange	3	348.42	116.14	0.5068	0.68493
nightshift	1	3.06	3.06	0.0134	0.90985
slp30	2	1432.04	716.02	3.1243	0.08086
slpmed	2	69.35	34.67	0.1513	0.86121
under2care	1	2.53	2.53	0.0111	0.91799
caretoddler	1	831.88	831.88	3.6298	0.08099
workload_weight	2	566.24	283.12	1.2354	0.32520
after_work	2	46.76	23.38	0.1020	0.90378
job_sweat	1	8.04	8.04	0.0351	0.85452
playsports	2	168.59	84.30	0.3678	0.69978
vitamins	1	1150.11	1150.11	5.0184	0.04478
sleepclinic	1	73.65	73.65	0.3214	0.58124
highbp	1	88.35	88.35	0.3855	0.54629
prior_pregnancy	1	0.38	0.38	0.0016	0.96827
paidjob	1	689.96	689.96	3.0106	0.10831
race	2	570.83	285.41	1.2454	0.32251
Residuals	12	2750.15	229.18		

B

Weekday Cortisol ANOVA Table

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
alarmclock	1	0.000	0.000	0.0000	0.99750
shiftchange	3	11.760	3.920	0.6461	0.60022
nightshift	1	29.995	29.995	4.9436	0.04615
slp30	2	3.724	1.862	0.3069	0.74131
slpmed	2	16.386	8.193	1.3503	0.29585
under2care	1	0.662	0.662	0.1091	0.74689
caretoddler	1	11.031	11.031	1.8181	0.20244
workload_weight	2	2.783	1.391	0.2293	0.79847
after_work	2	8.873	4.436	0.7312	0.50161
job_sweat	1	35.271	35.271	5.8133	0.03285
playsports	2	2.657	1.329	0.2190	0.80650
vitamins	1	2.317	2.317	0.3819	0.54814
sleepclinic	1	3.364	3.364	0.5544	0.47087
highbp	1	1.964	1.964	0.3237	0.57988
prior_pregnancy	1	22.684	22.684	3.7386	0.07710
paidjob	1	5.192	5.192	0.8558	0.37315
race	2	4.492	2.246	0.3701	0.69825
Residuals	12	72.809	6.067		

C

Weekend Midpoint Sleep ANOVA Table

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
alarmclock	1	0.0111	0.0111	0.0121	0.913270
shiftchange	3	11.4021	3.8007	4.1713	0.017576
nightshift	1	0.0588	0.0588	0.0645	0.801903
slp30	2	13.8076	6.9038	7.5770	0.003138
slpmed	2	4.6580	2.3290	2.5561	0.100423
under2care	1	4.6059	4.6059	5.0550	0.034906
caretoddler	1	0.0913	0.0913	0.1002	0.754552
workload_weight	2	9.1824	4.5912	5.0389	0.015791
after_work	2	0.3415	0.1707	0.1874	0.830426
job_sweat	1	3.2072	3.2072	3.5200	0.073963
playsports	2	0.3368	0.1684	0.1848	0.832523
vitamins	1	9.3564	9.3564	10.2688	0.004089
sleepclinic	1	0.1563	0.1563	0.1716	0.682709
highbp	1	11.9823	11.9823	13.1506	0.001493
prior_pregnancy	1	1.1884	1.1884	1.3043	0.265707
paidjob	1	0.3532	0.3532	0.3876	0.539945
Residuals	22	20.0454	0.9112		

D

Weekend Melatonin ANOVA Table

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
alarmclock	1	839.7	839.74	4.6555	0.044714
shiftchange	3	213.5	71.17	0.3945	0.758454
nightshift	1	2.6	2.58	0.0143	0.906126
slp30	2	790.6	395.32	2.1916	0.140649
slpmed	2	55.2	27.59	0.1529	0.859284
under2care	1	4.0	4.01	0.0222	0.883093
caretoddler	1	1763.5	1763.51	9.7768	0.005827
workload_weight	2	499.5	249.75	1.3846	0.275852
after_work	2	210.4	105.19	0.5832	0.568318
job_sweat	1	207.6	207.59	1.1509	0.297543
playsports	2	408.4	204.22	1.1322	0.344240
vitamins	1	1391.5	1391.49	7.7144	0.012421
sleepclinic	1	110.2	110.19	0.6109	0.444610
highbp	1	122.8	122.77	0.6806	0.420165
prior_pregnancy	1	10.7	10.65	0.0591	0.810746
paidjob	1	694.4	694.39	3.8497	0.065407
race	2	573.7	286.87	1.5904	0.231188
Residuals	18	3246.8	180.38		

E

Weekend Cortisol ANOVA Table

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
alarmclock	1	0.121	0.121	0.0198	0.88926
shiftchange	3	22.221	7.407	1.2103	0.32831
nightshift	1	31.956	31.956	5.2217	0.03186
slp30	2	3.492	1.746	0.2853	0.75443
slpmed	2	8.939	4.469	0.7303	0.49259
under2care	1	0.515	0.515	0.0841	0.77441
caretoddler	1	16.815	16.815	2.7476	0.11098
workload_weight	2	5.254	2.627	0.4292	0.65612
after_work	2	6.674	3.337	0.5453	0.58701
job_sweat	1	14.868	14.868	2.4294	0.13273
playsports	2	1.227	0.613	0.1002	0.90501
vitamins	1	0.056	0.056	0.0092	0.92457
sleepclinic	1	1.361	1.361	0.2224	0.64163
highbp	1	0.272	0.272	0.0445	0.83481
prior_pregnancy	1	9.475	9.475	1.5482	0.22593
paidjob	1	0.005	0.005	0.0008	0.97729
Residuals	23	140.756	6.120		

F

Table 6: ANOVA tables for the second trimester: A for weekday midpoint sleep time; B for weekday melatonin level; C for weekday cortisol level; D for weekend midpoint sleep time; E for weekend melatonin level; and F for weekend cortisol level.

3.3 Variable Selection and Linear Regression

3.2.1 Distribution of response variables

The histogram of midpoint sleep time is shown in Figure 4. Midpoint sleep time in non-pregnancy was approximately normally distributed with mean ($\bar{x}$) of 2.99 and standard deviation (s) of 2.28. It was dramatically higher in the first trimester ($\bar{x}$ =2.61, s=1.81) and kept a similar level in the second trimester ($\bar{x}$=2.78, s=1.91). Pregnant women dropped their melatonin value in the third trimester ($\bar{x}$=2.99, s=1.29) to the level of baseline, and their melatonin peak values showed a closer approximation normally distributed, compared to distributions in other periods.

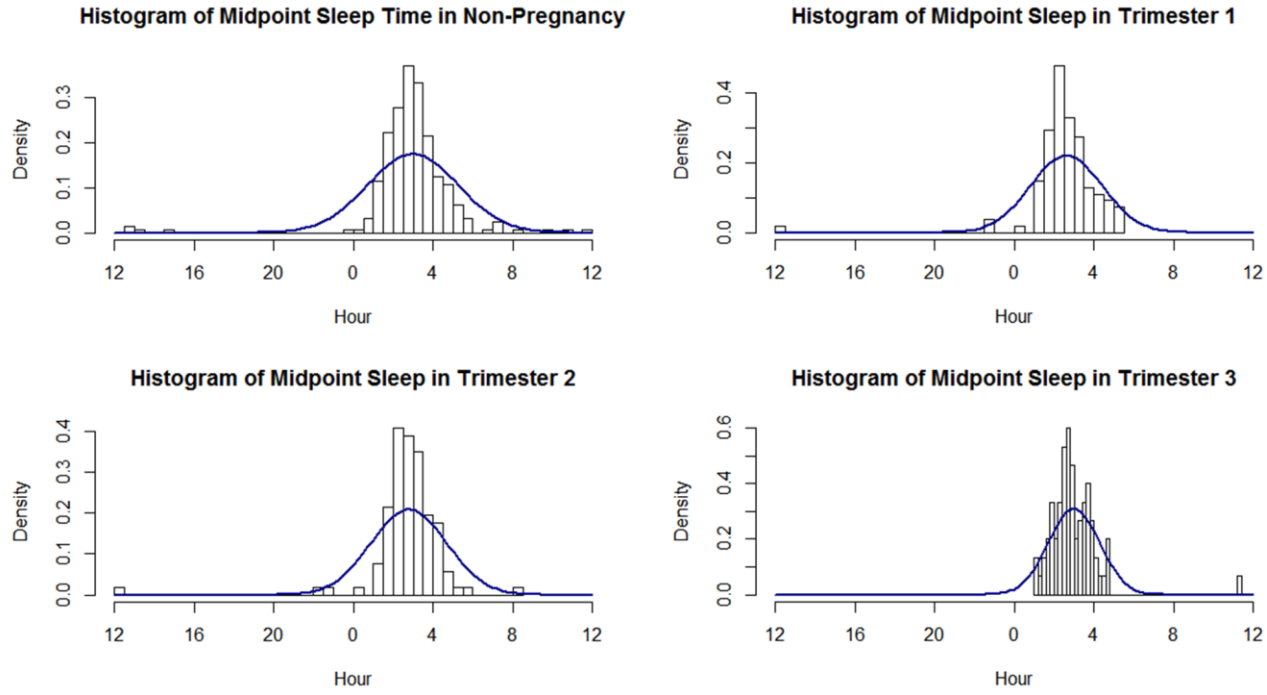

Figure 4: Histograms of midpoint sleep time in non-pregnancy and three trimesters. Blue line shows fitted a normal distribution.

The histogram of melatonin peak is shown in Figure 5. Melatonin peak in non-pregnancy was left shifted with mean of 27.87 and standard deviation of 22.40. It was dramatically increased in the first trimester ($\bar{x}$ =46.39, s=137.68) and kept similar level in the second trimester ($\bar{x}$=46.57,

s=163.10). Pregnant women dropped their melatonin value in the third trimester ($\bar{x}$=27.89, s=14.37) to the level of baseline, and their melatonin peak values showed better approximation to the normal distribution, comparing to distributions in other periods. A logarithm transformation was applied to the melatonin peak values in the four periods. It showed marked improved towards being normally distributed.

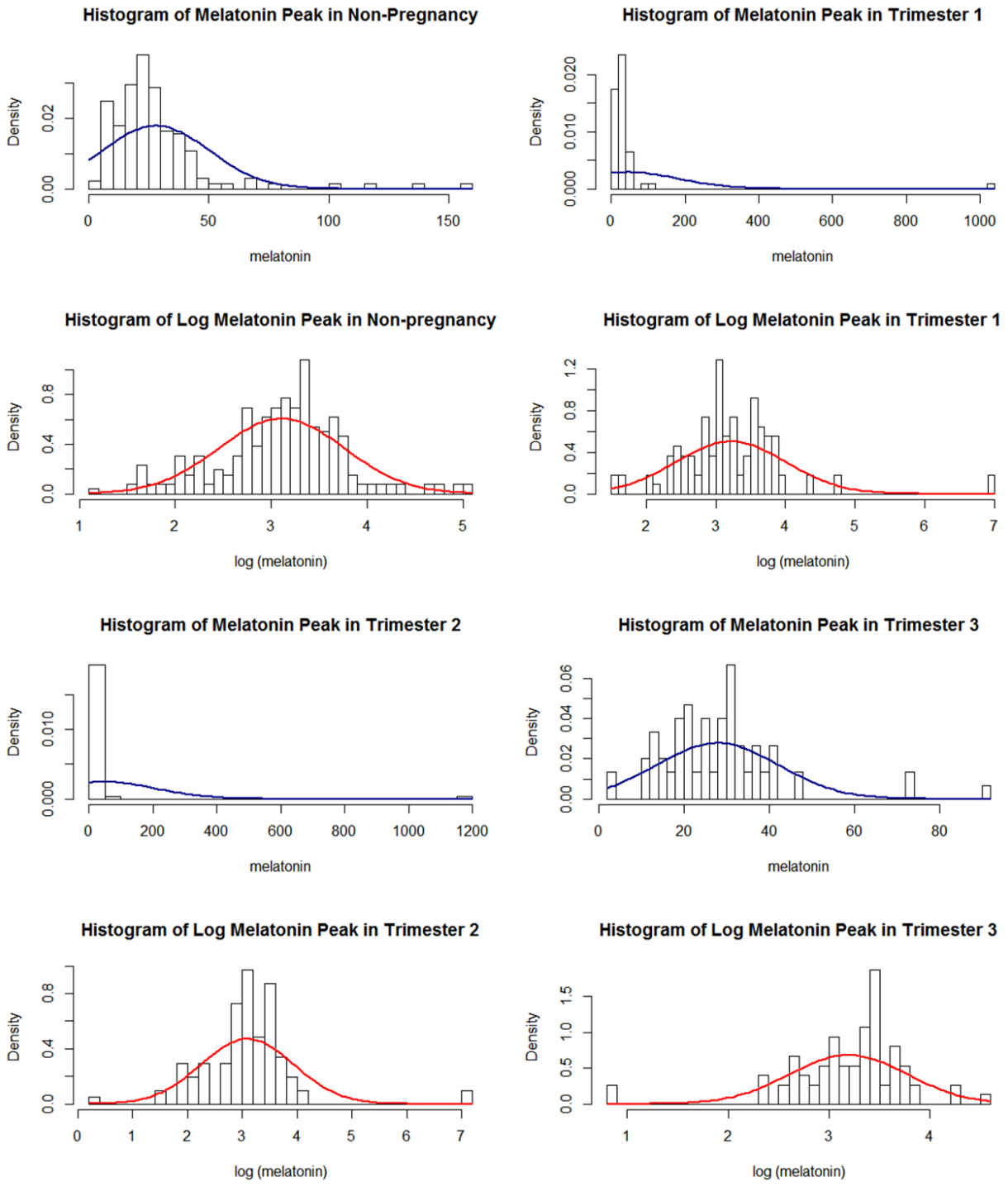

Figure 5: Histograms of melatonin peak value in non-pregnancy and three trimesters. For each period, the upper graph shows melatonin peak value, and blue line shows a fitted normal distribution. And the lower graph of each period shows a logarithm of melatonin peak value with a red fitted normal distribution.

The histogram of cortisol peak is showed in Figure 6. Cortisol peak in non-pregnancy had a left shifted distribution with $\bar{x}$=4.62 and standard deviation s=2.48. It was slightly decreased in the first trimester ($\bar{x}$=4.23, s=1.80) and became higher in the second trimester ($\bar{x}$=4.90, s=3.59). Then cortisol peak value kept relatively high level in the third trimester ($\bar{x}$=4.88, s=2.35) level. Logarithm was done to melatonin peak in the four periods. It showed marked improved towards bing normallly distributed with the logarithm transformation, especially in non-pregnancy, trimester 1 and trimester 2.

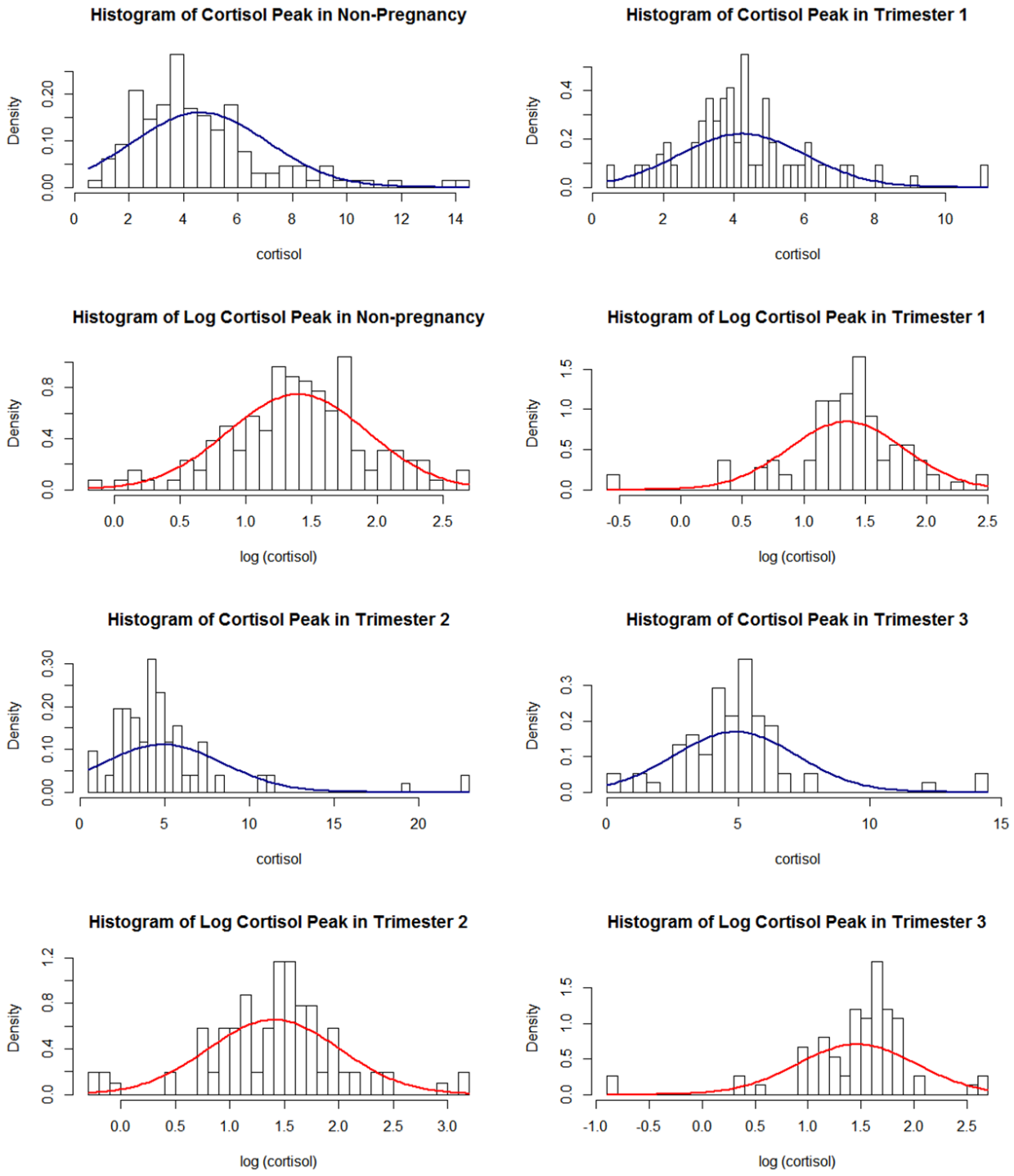

Figure 6: Histograms of cortisol peak value in non-pregnancy and three trimesters. For each period, the upper graph shows cortisol peak value, and blue line shows a fitted normal distribution. And the lower graph of each period shows a logarithm of cortisol peak value with a red fitted normal distribution.

3.3.1 Correlation of factors for all periods

After processing the ANOVA analysis above, correlation tables were generated address the relationships among factors and their correlation with outcome variables.

In before pregnancy data set, there were several features having strong correlation. Such as, *nightshift* and *shiftchange* shared large positive correlation, same as *workload_weight* with *job_sweat, slp30* with *slpmed* and *under2care, caretoddler* with *prior_pregnancy*. Therefore, they can be considered as the same group. *weekday* had relatively large negative correlation with midpoint sleep time, while *slp30* was shown as the second large correlation in absolute value (Figure 7). The melatonin peak value had negative correlation with *alarmclock* and *prior_pregnancy,* while had positive correlation with *vitamins* and *slpmed* (Figure 7). The cortisol peak value showed relative obvious correlation with *playsports*, *paidjob*, *highbp,* and *caretoddler* (Figure 7).

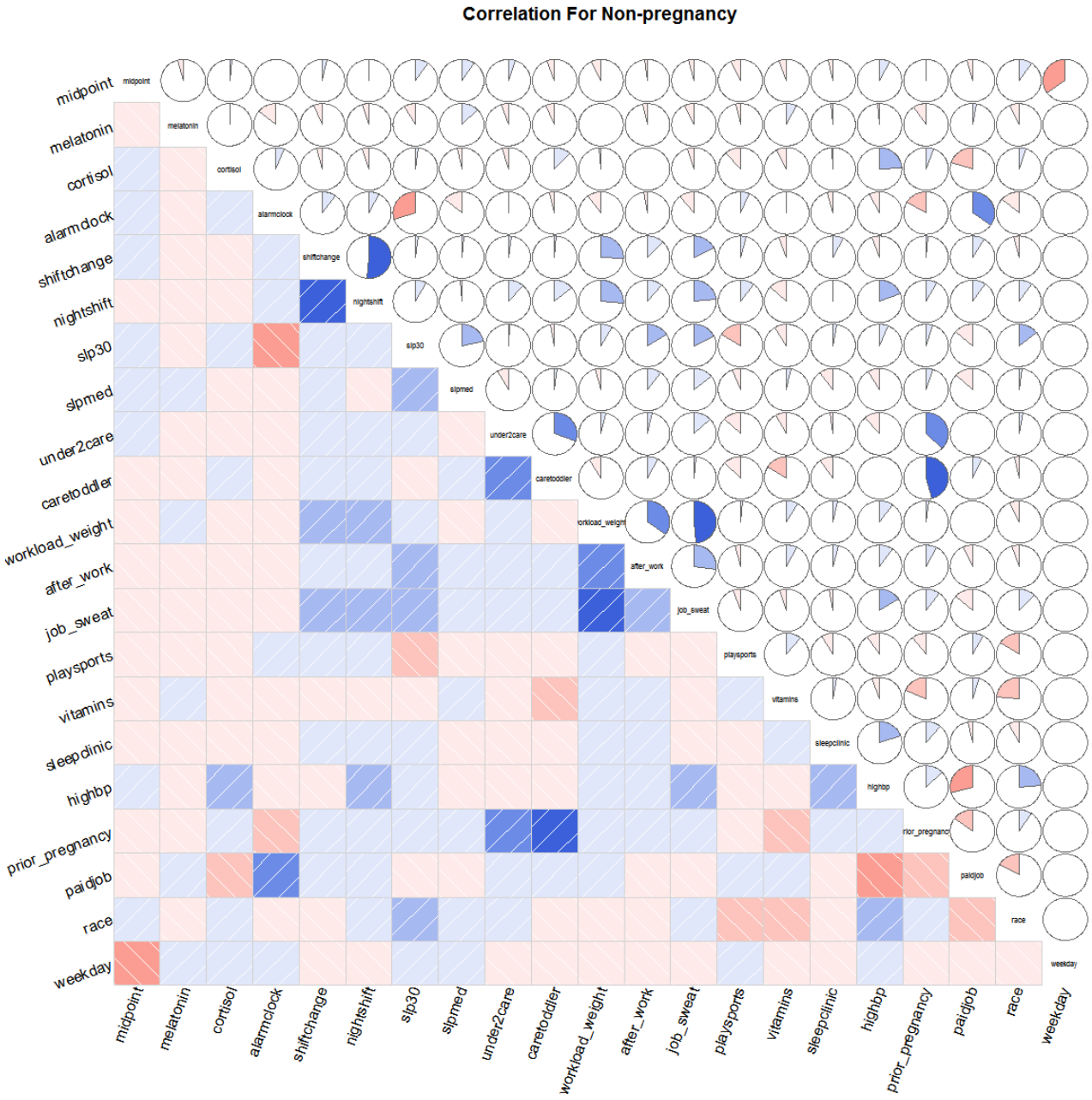

Figure 7: The Correlation between factors during non-pregnancy. Color (blue for positive values, red for negative values) is used to encode the sign of the correlation, where the intensity of color increases uniformly as the correlation value moves away from 0. Upper-right half of the figure uses pie charts, which demonstrate the quantification of the correlation.

During the first trimester (Figure 8), midpoint sleep time was highly positive correlated with *shiftchange* while negative correlated wit*h prior_pregnancy* and *weekday*. In the term of melatonin, there was strong positive correlation with *slpmed* and *race*, as well as *alarmclock* and

paidjob. For cortisol, from Figure 8, we can see that it had larger positive correlation with *vitamins* and *job_sweat*, while had negative correlation with *race*, *slp30* and *slpmed*.

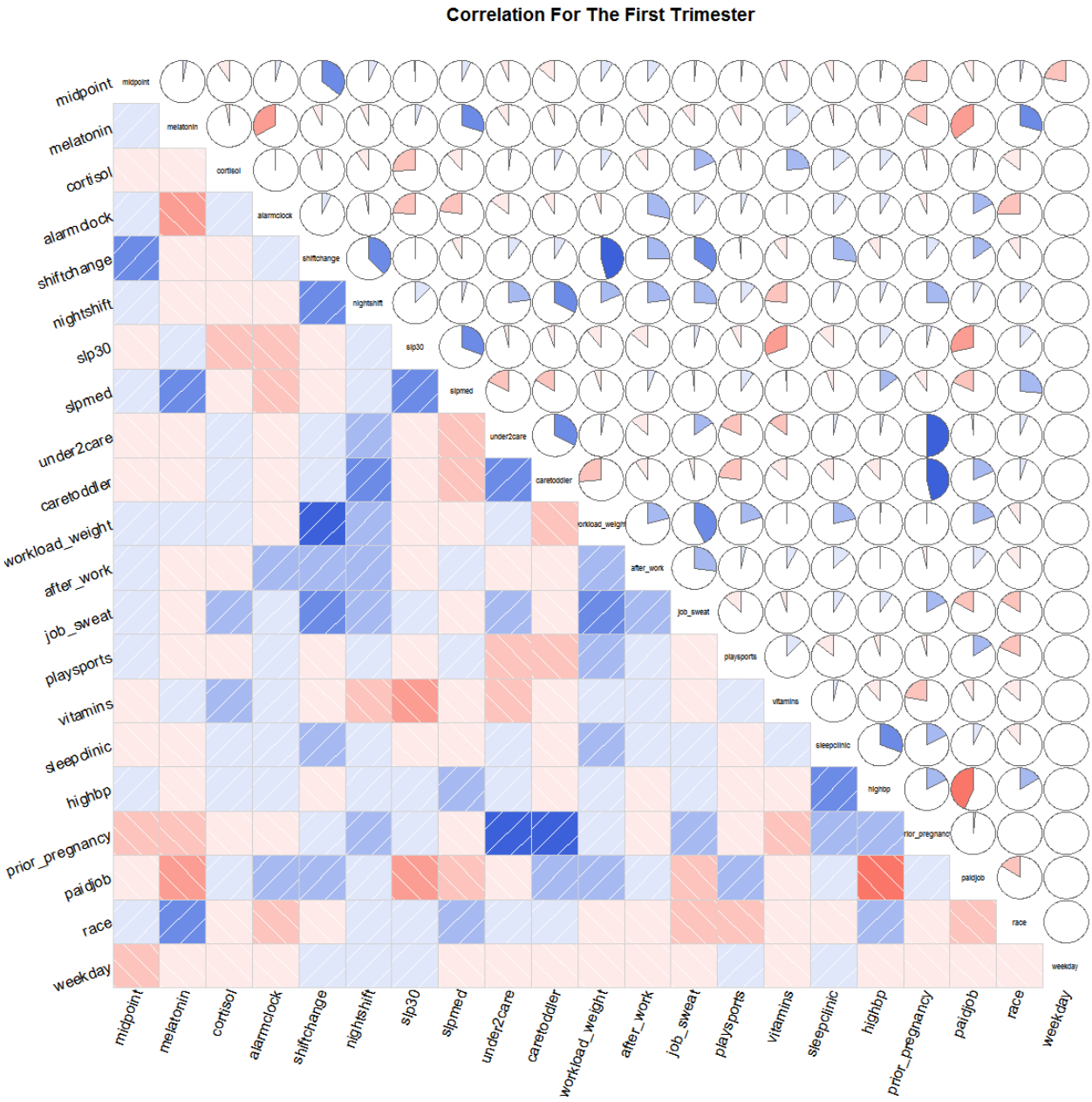

Figure 8: The Correlation between factors during the first trimester. Color (blue for positive values, red for negative values) is used to encode the sign of the correlation, where the intensity of color increases uniformly as the correlation value moves away from 0. Upper-right half of the figure uses pie charts, which demonstrate the quantification of the correlation.

Figure 9 shows the correlations between each factor in the second trimester. Mid sleep time also showed strong negative correlation with *weekday, paidjob* and *prior_pregnancy,* and positive with *shiftchange, slpmed, highbp* and *race*. Melatonin in this period was still with high positive correlation with *vitamins* and *race*, and also with *shiftchange*. Negative correlation factor with melatonin were *alarmclock, job_sweat* and *paidjob.* Cortisol had the strongest negative correlation with *sleepclinic, slp30, prior_pregnancy* and *caretoddler*, while it had positive correlation with *alarmclock, shiftchange* and *sleepclininc*.

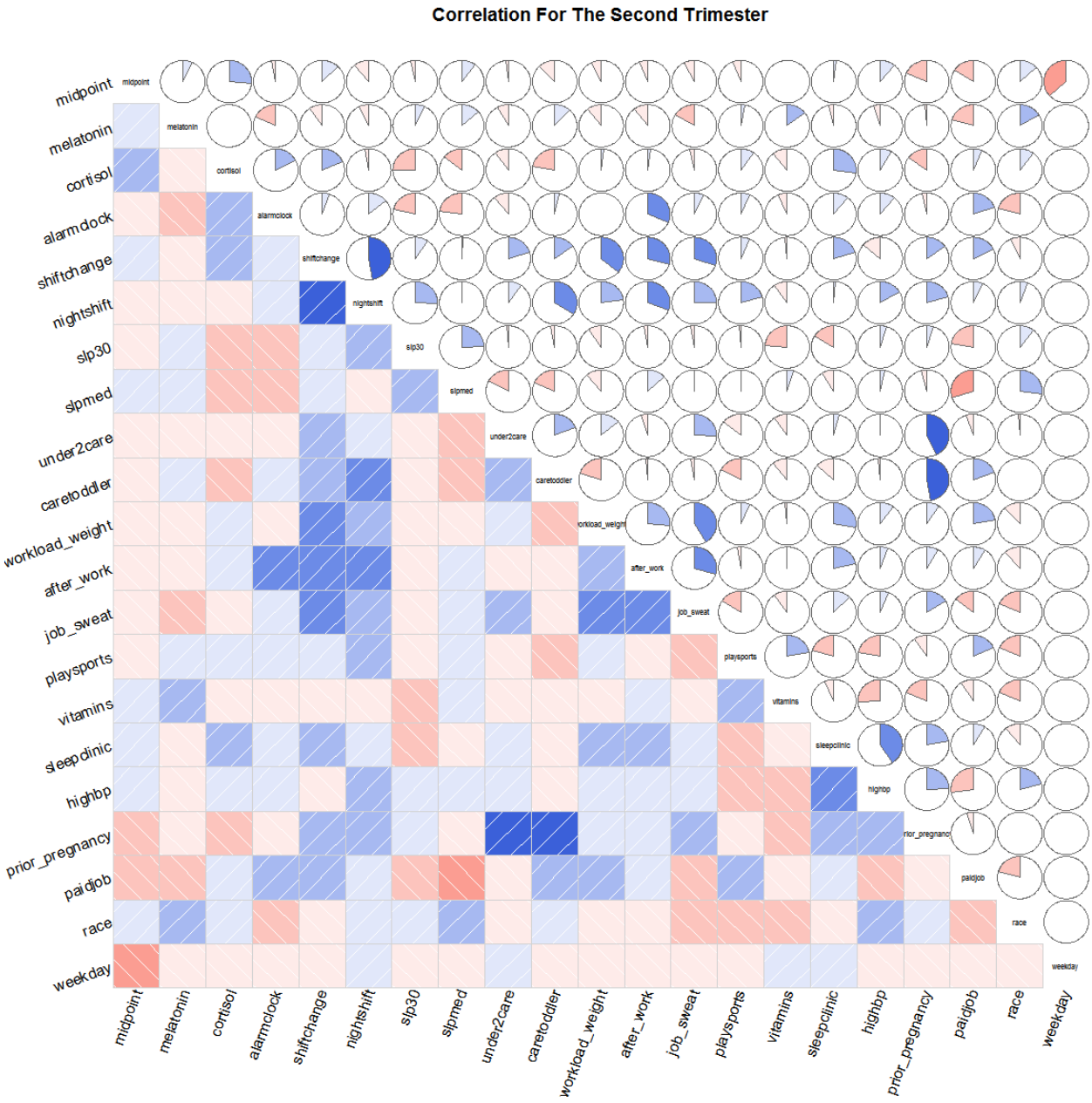

Figure 9: The Correlation between factors during the second trimester. Color (blue for positive values, red for negative values) is used to encode the sign of the correlation, where the intensity of color increases uniformly as the correlation value moves away from 0. Upper-right half of the figure uses pie charts, which demonstrate the quantification of the correlation.

Similar as previous figures, in the third trimester, the midpoint sleep time showed strong negative correlation with *weekdays* and *paidjob*, as well as *caretoddler* and *under2care* which were not as strong as the previous 2 factor (Figure 10). *Slp30* and *slpmed* showed to be positive

correlated with the midpoint sleep time. Melatonin showed negative correlation with *caretoddler* and *prior_pregnancy*, and positive correlation with *workload_weight* and *vitamins*. Cortisol, on the other hand, seemed to have strong negative correlation with *nightshift*, *caretoddler*, *shiftchange*, *paidjob* and *slpmed*; and had negative correlation with *vitamins*.

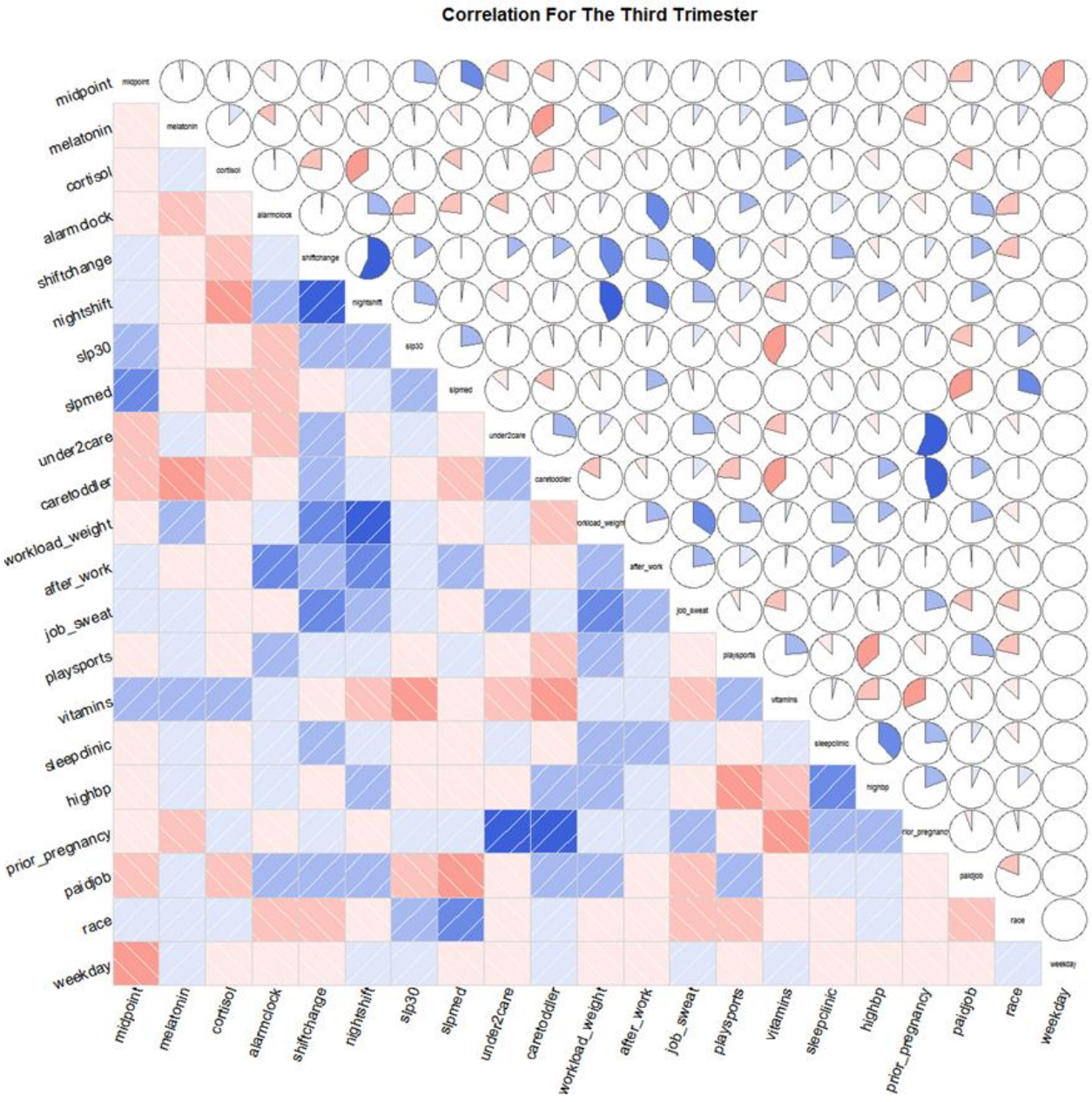

Figure 10: The Correlation between factors during the third trimester. Color (blue for positive values, red for negative values) is used to encode the sign of the correlation, where the intensity

of color increases uniformly as the correlation value moves away from 0. Upper-right half of the figure uses pie charts, which demonstrate the quantification of the correlation.

3.3.2 Linear regression for midpoint sleep time

After considering all factors and all correlations in Section 3.2.1, four regression models of midpoint sleep time for non-pregnancy, T1, T2, and T3 were built[1] as below:

```
  Model1<-lm(midpoint ~ weekday + under2care : slp30 + slp30 :
workload_weight + slp30, data=data_T0);

  Model2<-lm(midpoint ~ weekday + prior_pregnancy+paidjob + shiftchange +
workload_weight, data=data_T1)

  Model3<-lm(midpoint ~ weekday + paidjob + prior_pregnancy * workload_weight
+ shiftchange + highbp + slpmed, data=data_T2)

  Model4<-lm(slp30 + weekday + vitamins + workload_weight + job_sweat,
data=data_T3)
```

From Table 7, it is obvious that non-pregnant women, who took care of younger than 2 year-old children and cannot get to sleep within 30 minutes three times or more a week, and experienced 2.5 hours later midpoint sleep time than non-pregnant women who had not. However, women, who cannot get to sleep within 30 minutes three times or more in a week and had physically heavy workload, were significantly different at 3.5 hours earlier midpoint sleep time than women who got to sleep quickly and had light workload job. Furthermore, women who cannot get to sleep within 30 minutes three times or more in a week showed a 1.7 hours later midpoint sleep time than women who had no problem to get to sleep with t-value = 1.759 and p-value = 0.08. The whole model showed a 0.1567 adjusted-R^2, but the residuals-fitted plot and the Q-Q plot showed it was approximately normally distributed with a few outliers (Figure 11).

[1] Multiple imputation of missing data using Fully Conditional Specification (FCS) implemented by the MICE algorithm as described in Van Buuren and Groothuis-Oudshoorn (2011) were also applied to all models, but it didn't approve models much. Therefore, data without imputation was used for all following models.

Non-Pregnancy -Model 1

Coefficients:

	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	15.6001	0.4866	32.062	< 2e-16
weekday1	-1.4606	0.2603	-5.612	5.38e-08
slp301	0.2846	0.5919	0.481	0.63113
slp302	1.7135	0.9741	1.759	0.07979
under2care1:slp300	-0.2489	0.5537	-0.449	0.65348
under2care1:slp301	0.7462	0.4671	1.598	0.11141
under2care1:slp302	2.5053	0.7821	3.203	0.00154
slp300:workload_weight2	-0.1653	0.5431	-0.304	0.76116
slp301:workload_weight2	-0.2584	0.4588	-0.563	0.57382
slp302:workload_weight2	-1.3269	0.9649	-1.375	0.17033
slp300:workload_weight3	-0.6210	0.6399	-0.970	0.33276
slp301:workload_weight3	-0.2390	0.4877	-0.490	0.62451
slp302:workload_weight3	-3.4522	1.0996	-3.139	0.00190

Residual standard error: 2.093 on 246 degrees of freedom
Multiple R-squared: 0.1959, Adjusted R-squared: 0.1567
F-statistic: 4.994 on 12 and 246 DF, p-value: 2.051e-07

Table 7: Regression result of midpoint sleep time for non-pregnancy. The "first" level is rolled into the intercept and all subsequent levels have a coefficient that represents their difference from the baseline.

In the first trimester, pregnant women during weekdays had a 1.1 hour earlier midpoint sleep time than on free days, as theoretically expected. Those who had a pregnancy before this one, who had paid jobs, who one had shift change during the past month, and and had heavy workload jobs experienced 0.7 less, 1.3 less, 1.3 more and 1.1 more hour midpoint sleep time respectively. The adjusted R^2 was 0.1457, and this was relatively good comparing to others that had been tried using other factors. The residuals-fitted plot and the Q-Q plot showed it was nearly normal distributed with a few outliers (Figure11).

Trimester 1 -Model 2

```
Coefficients:
                 Estimate Std. Error t value Pr(>|t|)
(Intercept)      16.78783    0.74619  22.498  < 2e-16
weekday1         -1.10707    0.32092  -3.450 0.000823
prior_pregnancy1 -0.70687    0.35454  -1.994 0.048901
paidjob1         -1.32553    0.63281  -2.095 0.038729
shiftchange1      1.30798    0.59337   2.204 0.029795
shiftchange2      0.86781    0.68146   1.273 0.205809
shiftchange3      0.88061    1.28315   0.686 0.494120
workload_weight2  0.06392    0.39680   0.161 0.872349
workload_weight3 -1.08250    0.53539  -2.022 0.045857
Residual standard error: 1.675 on 100 degrees of freedom
Multiple R-squared:  0.209,  Adjusted R-squared:  0.1457
F-statistic: 3.303 on 8 and 100 DF,  p-value: 0.002179
```

Table 8: Regression result of midpoint sleep time for trimester 1. The “first” level is rolled into the intercept and all subsequent levels have a coefficient that represents their difference from the baseline.

In the second trimester, *weekday*, *paidjob*, *prior_pregnancy*, *workload_weight* and *shiftchange* affected midpoint sleep time as similar as non-pregnancy. Women who had high blood pressure showed 1.4 hour later midpoint sleep time in this period as expected, but the p-value was high as 0.159. The adjusted R^2 was 0.2151, and this was relatively good comparing to others that had been tried using other factors. The residuals-fitted plot and the Q-Q plot showed it was near normally distributed with few notable outliers (Figure 11).

Trimester 2 -Model 3

```
Coefficients:
                                  Estimate Std. Error t value Pr(>|t|)
(Intercept)                       17.11982    0.87946  19.466  < 2e-16
weekday1                          -1.21569    0.33457  -3.634 0.000467
paidjob1                          -1.35865    0.70664  -1.923 0.057716
prior_pregnancy1                  -0.92845    0.75206  -1.235 0.220253
workload_weight2                  -0.06997    0.56006  -0.125 0.900855
workload_weight3                  -0.75699    0.74913  -1.010 0.315002
shiftchange1                       1.93873    0.70986   2.731 0.007609
shiftchange2                       1.87315    0.72370   2.588 0.011264
shiftchange3                       2.64421    0.86244   3.066 0.002873
highbp1                            1.38592    0.97559   1.421 0.158927
slpmed1                           -0.91493    0.66355  -1.379 0.171403
slpmed2                            1.99858    1.26825   1.576 0.118608
prior_pregnancy1:workload_weight2 -0.34515    0.89849  -0.384 0.701788
prior_pregnancy1:workload_weight3 -1.10726    1.04838  -1.056 0.293751

Residual standard error: 1.694 on 89 degrees of freedom
Multiple R-squared:  0.3152, Adjusted R-squared:  0.2151
F-statistic: 3.151 on 13 and 89 DF,  p-value: 0.0006644
```

Table 9: Regression result of midpoint sleep time for trimester 2. The “first” level is rolled into the intercept and all subsequent levels have a coefficient that represents their difference from the baseline.

In the third trimester, *slp30, weekday* and *workload_weight* had very similar effects to the midpoint sleep time in the model as before. However, *vitamins* and *job_sweat* appeared to be important in the model as well. Women who took vitamins or had experienced sweat at work significantly demonstrated an hour later midpoint sleep time. This model was fits well with a 0.5473 R^2 and 13.78 F-statistic. The residuals-fitted plot and the Q-Q plot showed it was close to normally distributed with a few outliers as showed in Figure 11.

Trimester 3 -Model 4

```
Coefficients:
                 Estimate Std. Error t value Pr(>|t|)
(Intercept)       13.9558     0.3040  45.915  < 2e-16
slp301             0.3786     0.2436   1.554 0.124865
slp302             1.6083     0.3193   5.037 3.81e-06
weekday1          -0.9286     0.2011  -4.617 1.82e-05
vitamins1          1.0857     0.2477   4.384 4.21e-05
workload_weight2   0.2857     0.2483   1.151 0.254027
workload_weight3  -1.4605     0.3708  -3.939 0.000197
job_sweat1         1.0799     0.2477   4.359 4.59e-05

Residual standard error: 0.8671 on 67 degrees of freedom
Multiple R-squared:  0.5901, Adjusted R-squared:  0.5473
F-statistic: 13.78 on 7 and 67 DF,  p-value: 6.595e-11
```

Table 10: Regression result of midpoint sleep time for trimester 3. The “first” level is rolled into the intercept and all subsequent levels have a coefficient that represents their difference from the baseline.

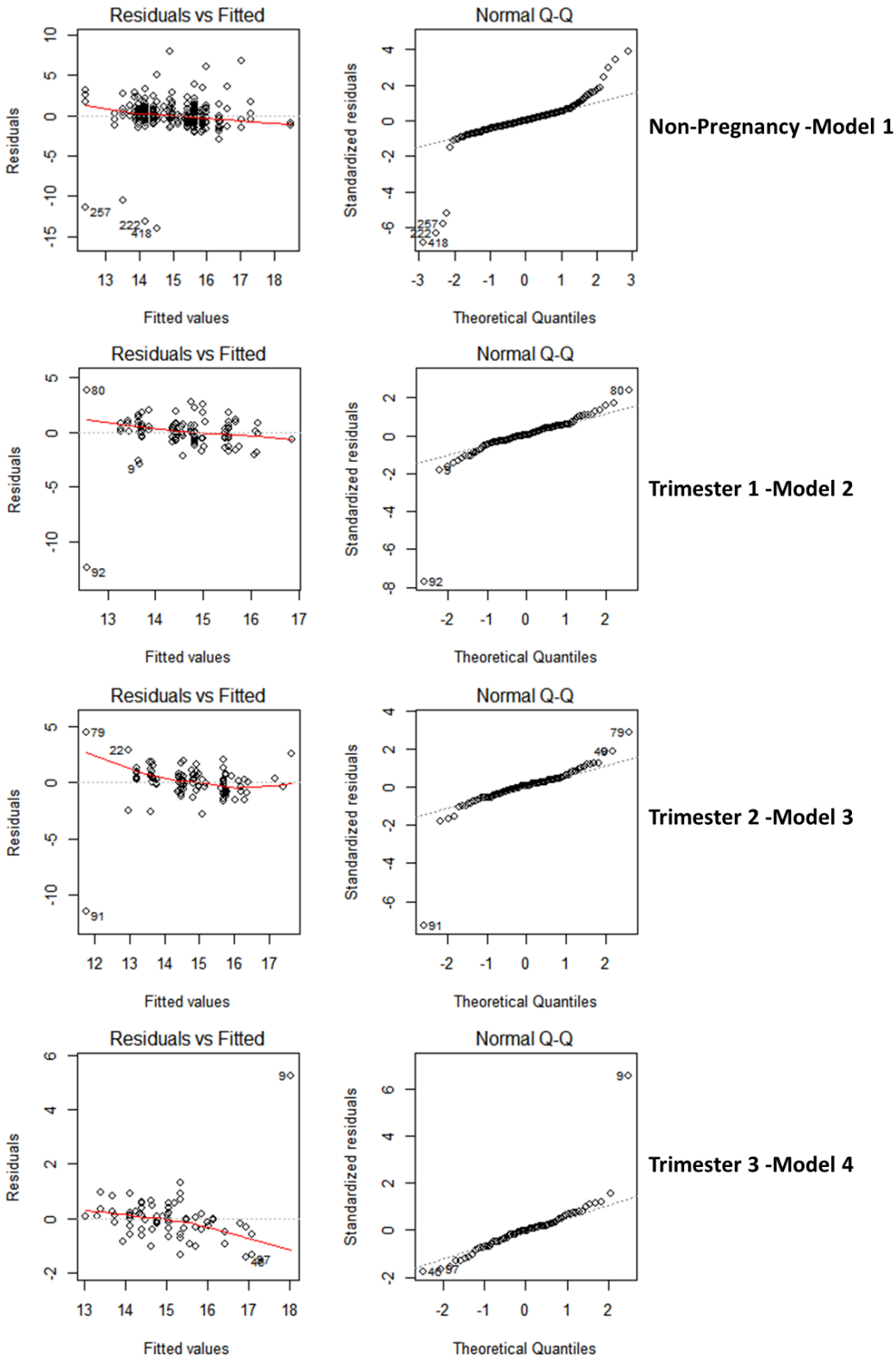

Figure 11: Useful plots of midpoint sleep time regression for all periods

Influence tests including outlier tests and leverage tests were done for the above four models (Figure 12). Outliers were identified by high absolute studentized residuals and influence by high Cook's distances. Leverages of models were identified by hat-values, where higher hat-value showed larger leverage. All such summaries for models are showed in Figure 12 with numbers and also quantified below:

Influences for Model 1 (non-pregnancy):

```
##         StudRes        Hat        CookD
## 90   0.09759043 0.17053304 0.0001512282
## 257 -6.20803459 0.11244186 0.3258497181
## 418 -7.63357737 0.04967473 0.1900551190
```

Influences for Model 2 (1st trimester):

```
##         StudRes        Hat        CookD
## 11  -0.04539457 0.50917823 0.0002399203
## 92 -12.13520020 0.09581019 0.7040510874
```

Influences for Model 3 (2nd trimester):

```
##          StudRes       Hat        CookD
## 91  -11.40533802 0.1311648 0.5724519758
## 132  -0.07028685 0.5097551 0.0003710663
```

Influences for Model 4 (3rd trimester):

```
##       StudRes       Hat      CookD
## 9  10.8740072 0.1523657 0.96616054
## 20  0.7273012 0.1986913 0.01651131
```

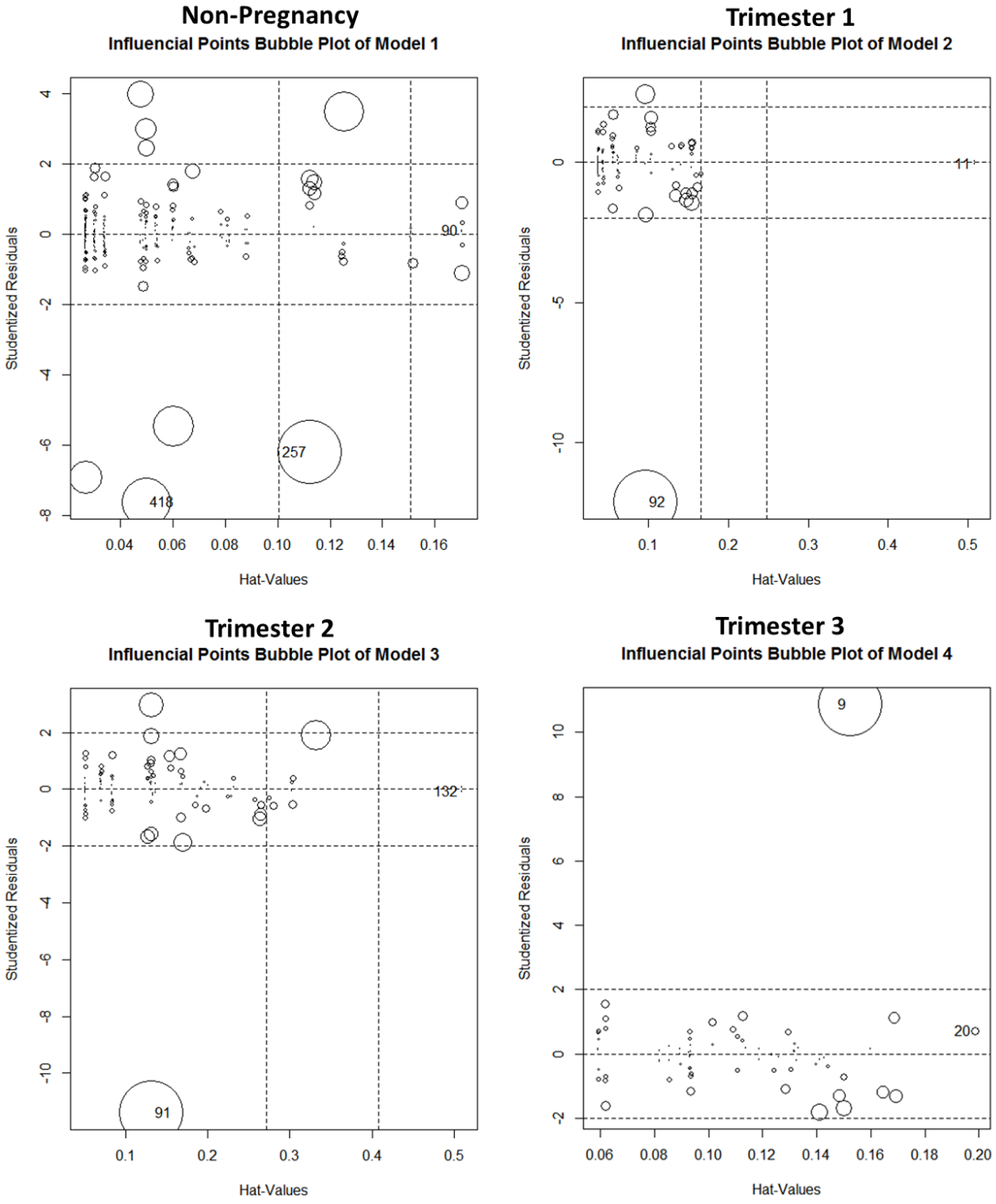

Figure 12: Influential points bubble plot of midpoint sleep time regression for four periods. Lager bubbles showed bigger influences.

Additional model specifications were constructed by remvoing all of the above identified influential points, but the resulting fits were not improved much as might expected with no sign changes or reasonable magnitude changes in the estimated coefficients, so the original models were kept.

3.3.3 Linear regression for melatonin peak value

After considering all factors and all correlations in Section 3.2.1, four regression models of log melatonin peak value for non-pregnancy, T1, T2, and T3 were built as below:

```
  Model5<-lm(log(melatonin) ~ slpmed:prior_pregnancy+prior_pregnancy + slp30
* highbp + paidjob * workload_weight + slp30 * playsports + workload_weight +
nightshift, data=data_T0)

  Model6<-lm(log(melatonin) ~ paidjob + alarmclock + prior_pregnancy *
nightshift + slpmed + slp30 * workload_weight, data=data_T1)

  Model7<-lm(log(melatonin) ~ prior_pregnancy * slp30 + race + paidjob +
alarmclock + workload_weight + highbp, data=data_T2)

  Model8<-lm(log(melatonin) ~ caretoddler + workload_weight+  playsports +
alarmclock:race + race, data=data_T3)
```

From the non-pregnancy regression result of model 5 (Table 11), women who had pregnancy before were shown to have 0.29 less log melatonin peak value with a p-value equal to 0.0006. Having the problem of getting to sleep within 30 minutes decreased melatonin peak value, and more times women experienced that problem dropped their melatonin more. There were some factors, including high blood pressure, paid jobs, and heavy workload weight, significantly increased log melatonin peak value by 0.84, 1.1, and 1.1 respectively. Women with nightshift had 0.17 less log melatonin peak value from the regression result. Surprisingly, the interaction between *paidjob* and normal/heavier *workload_weight* showed decreased log melatonin value, even though each factor by itself was increasing this value. The model had an adjusted R^2 value

0.28, and this was relatively good comparing to others that had been tried using other factors. The residuals-fitted plot and the Q-Q plot showed it was very close to normally distributed with a few outliers (Figure 13).

Non-Pregnancy -Model 5

```
Coefficients:
                           Estimate Std. Error t value Pr(>|t|)
(Intercept)                 2.53073    0.34577   7.319 3.89e-12
prior_pregnancy1           -0.28530    0.08246  -3.460 0.000641
slp301                     -0.47953    0.22278  -2.152 0.032377
slp302                     -0.77628    0.25181  -3.083 0.002295
highbp1                     0.84024    0.41292   2.035 0.042981
paidjob1                    1.12080    0.30035   3.732 0.000238
workload_weight2            1.55598    0.36096   4.311 2.39e-05
workload_weight3            2.05576    0.43235   4.755 3.46e-06
playsports1                -0.43724    0.21733  -2.012 0.045374
playsports2                -0.31433    0.20570  -1.528 0.127820
nightshift1                -0.16633    0.08738  -1.903 0.058198
slpmed1:prior_pregnancy0    0.18437    0.15575   1.184 0.237693
slpmed2:prior_pregnancy0   -0.37521    0.43956  -0.854 0.394188
slpmed1:prior_pregnancy1    0.05888    0.22151   0.266 0.790610
slpmed2:prior_pregnancy1    0.61591    0.17959   3.430 0.000713
slp301:highbp1             -1.69374    0.45724  -3.704 0.000264
slp302:highbp1              1.17879    0.59331   1.987 0.048100
paidjob1:workload_weight2  -1.51545    0.37485  -4.043 7.15e-05
paidjob1:workload_weight3  -1.95095    0.44637  -4.371 1.86e-05
slp301:playsports1          0.77362    0.27575   2.806 0.005442
slp302:playsports1          0.15772    0.40790   0.387 0.699355
slp301:playsports2          0.39476    0.24645   1.602 0.110548
slp302:playsports2          0.38873    0.30209   1.287 0.199424
Residual standard error: 0.5547 on 236 degrees of freedom
Multiple R-squared:  0.3479, Adjusted R-squared:  0.2871
F-statistic: 5.722 on 22 and 236 DF,  p-value: 8.606e-13
```

Table 11: Regression result of log (melatonin) for non-pregnancy. The “first” level is rolled into the intercept and all subsequent levels have a coefficient that represents their difference from the baseline.

In the first trimester, *prior_pregnancy*, *slp30*, *nightshift*, as well as interaction between *slp30* and *workload_weight*, affected the outcome similarly as non-pregnancy period. While new factors, such as using alarm clock and sleeping medication, turned out to effect melatonin peak value as well. With adjusted R^2 equals to 0.5394 and F-statistic equals to 9.43, this model was relatively good comparing to others that had been tried adding other factors. The residuals-fitted

plot and the Q-Q plot showed it was very close to normally distributed with few outliers (Figure 13).

Trimester -Model 6

```
Coefficients:
                             Estimate Std. Error t value Pr(>|t|)
(Intercept)                   4.67278    0.32715  14.283  < 2e-16
paidjob1                     -0.12303    0.23804  -0.517 0.606503
alarmclock1                  -1.24228    0.16800  -7.395 6.10e-11
prior_pregnancy1             -0.54679    0.14108  -3.876 0.000198
nightshift1                  -1.19799    0.52169  -2.296 0.023901
slpmed1                       1.16757    0.31590   3.696 0.000370
slpmed2                      -1.55177    0.51935  -2.988 0.003592
slp301                       -0.03902    0.22295  -0.175 0.861446
slp302                       -2.40970    0.52289  -4.608 1.29e-05
workload_weight2             -0.21209    0.21836  -0.971 0.333923
workload_weight3              0.33158    0.23683   1.400 0.164827
prior_pregnancy1:nightshift1  1.30577    0.53965   2.420 0.017480
slp301:workload_weight2       0.39359    0.29893   1.317 0.191197
slp302:workload_weight2       3.10610    0.65401   4.749 7.39e-06
slp301:workload_weight3      -0.45051    0.33069  -1.362 0.176380
slp302:workload_weight3       1.87139    0.67990   2.752 0.007112

Residual standard error: 0.5393 on 93 degrees of freedom
Multiple R-squared:  0.6033, Adjusted R-squared:  0.5394
F-statistic:  9.43 on 15 and 93 DF,  p-value: 5.298e-13
```

Table 12: Regression result of log (melatonin) for trimester 1. The “first” level is rolled into the intercept and all subsequent levels have a coefficient that represents their difference from the baseline.

In the second trimester, women had pregnancy before showed 0.5 a higher log melatonin peak value than those not. Black or African American demonstrated 0.13 a lower outcome than white people, but the p-value was high as 0.159, so we cannot conclude a different. Women who had high blood pressure showed 1.2 less log melatonin peak in this period as expected. Other factors had very similar effects as had previously happened. The adjusted R^2 was 0.3317, and this was relatively good comparing to others that had been tried using other factors. The residuals-fitted plot and the Q-Q plot showed it was approximately normally distributed with a few outliers (Figure 13).

Trimester 2 -Model 7

```
Coefficients:
                        Estimate Std. Error t value Pr(>|t|)
(Intercept)               3.3646     0.4168   8.073 2.88e-12
prior_pregnancy1          0.5003     0.2460   2.034 0.044927
slp301                    0.4287     0.2350   1.824 0.071494
slp302                    0.1034     0.3180   0.325 0.745731
race2                    -0.1289     0.2298  -0.561 0.576137
race3                     0.8588     0.2671   3.215 0.001809
paidjob1                 -0.5797     0.3065  -1.891 0.061814
alarmclock1              -0.4493     0.2089  -2.151 0.034155
workload_weight2          0.7339     0.1984   3.699 0.000372
workload_weight3          0.5151     0.2115   2.435 0.016859
highbp1                  -1.1883     0.4638  -2.562 0.012067
prior_pregnancy1:slp301  -1.0856     0.3703  -2.932 0.004275
prior_pregnancy1:slp302  -0.6513     0.4795  -1.358 0.177776

Residual standard error: 0.692 on 90 degrees of freedom
Multiple R-squared:  0.4103, Adjusted R-squared:  0.3317
F-statistic: 5.218 on 12 and 90 DF,  p-value: 1.384e-06
```

Table 13: Regression result of log (melatonin) for trimester 2. The “first” level is rolled into the intercept and all subsequent levels have a coefficient that represents their difference from the baseline.

In the third trimester, a pregnant woman who took care one or more toddlers had a decreased log melatonin peak value by 0.44. Other factors were very similar with before. This model was showed reasonable fit with a 0.2161 R^2 and 3.041 F-statistic. The residuals-fitted plot and the Q-Q plot showed it was approximately normally distributed with a few outliers as showed in Figure 13.

Trimester 3 -Model 8

```
Coefficients:
                  Estimate Std. Error t value Pr(>|t|)
(Intercept)        3.10543    0.37361   8.312 9.09e-12
caretoddler1      -0.43957    0.20485  -2.146   0.0357
workload_weight2  -0.17275    0.15387  -1.123   0.2658
workload_weight3   0.04101    0.18984   0.216   0.8296
playsports1        0.10117    0.34844   0.290   0.7725
playsports2        0.49081    0.32534   1.509   0.1363
race2             -0.43965    0.32778  -1.341   0.1846
race3              1.46706    0.56155   2.613   0.0112
alarmclock1:race1 -0.20608    0.19878  -1.037   0.3038
alarmclock1:race2  0.35537    0.37360   0.951   0.3451
alarmclock1:race3 -1.41078    0.56803  -2.484   0.0156

Residual standard error: 0.5169 on 64 degrees of freedom
Multiple R-squared:  0.3221, Adjusted R-squared:  0.2161
F-statistic: 3.041 on 10 and 64 DF,  p-value: 0.003286
```

Table 14: Regression result of log (melatonin) for trimester 3. The “first” level is rolled into the intercept and all subsequent levels have a coefficient that represents their difference from the baseline.

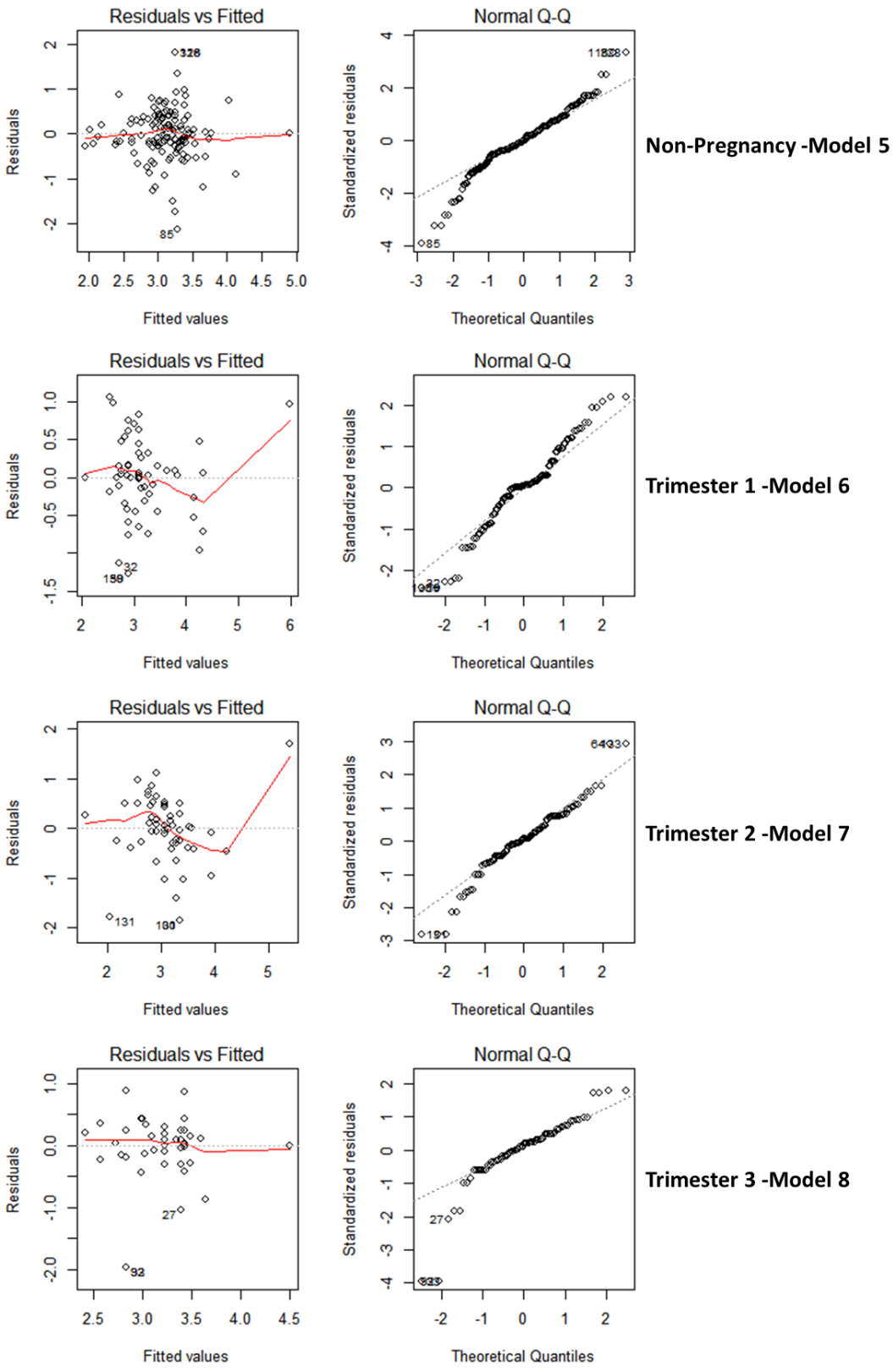

Figure 13: Useful plots of log (melatonin) regression for all periods

All influences for models were showed in Figure 14 with numbers and also quantified below:

Influences for Model 5 (non-pregnancy):

```
##       StudRes        Hat        CookD
## 85  -4.035449 0.02808764 1.921718e-02
## 119  0.000000 0.50000000 9.376447e-31
## 389 -1.878982 0.23581192 4.686525e-02
```

Influences for Model 6 (1st trimester):

```
##      StudRes       Hat        CookD
## 49 -2.234483 0.3323365 1.489352e-01
## 59 -2.489842 0.0500756 1.934351e-02
## 75  0.000000 0.5000000 6.557301e-31
```

Influences for Model 7 (2nd trimester):

```
##       StudRes        Hat        CookD
## 85  -4.035449 0.02808764 1.921718e-02
## 119  0.000000 0.50000000 9.376447e-31
## 389 -1.878982 0.23581192 4.686525e-02
```

Influences for Model 8 (3rd trimester):

```
##       StudRes        Hat     CookD
## 33  -4.524775 0.07948619 0.1232235
```

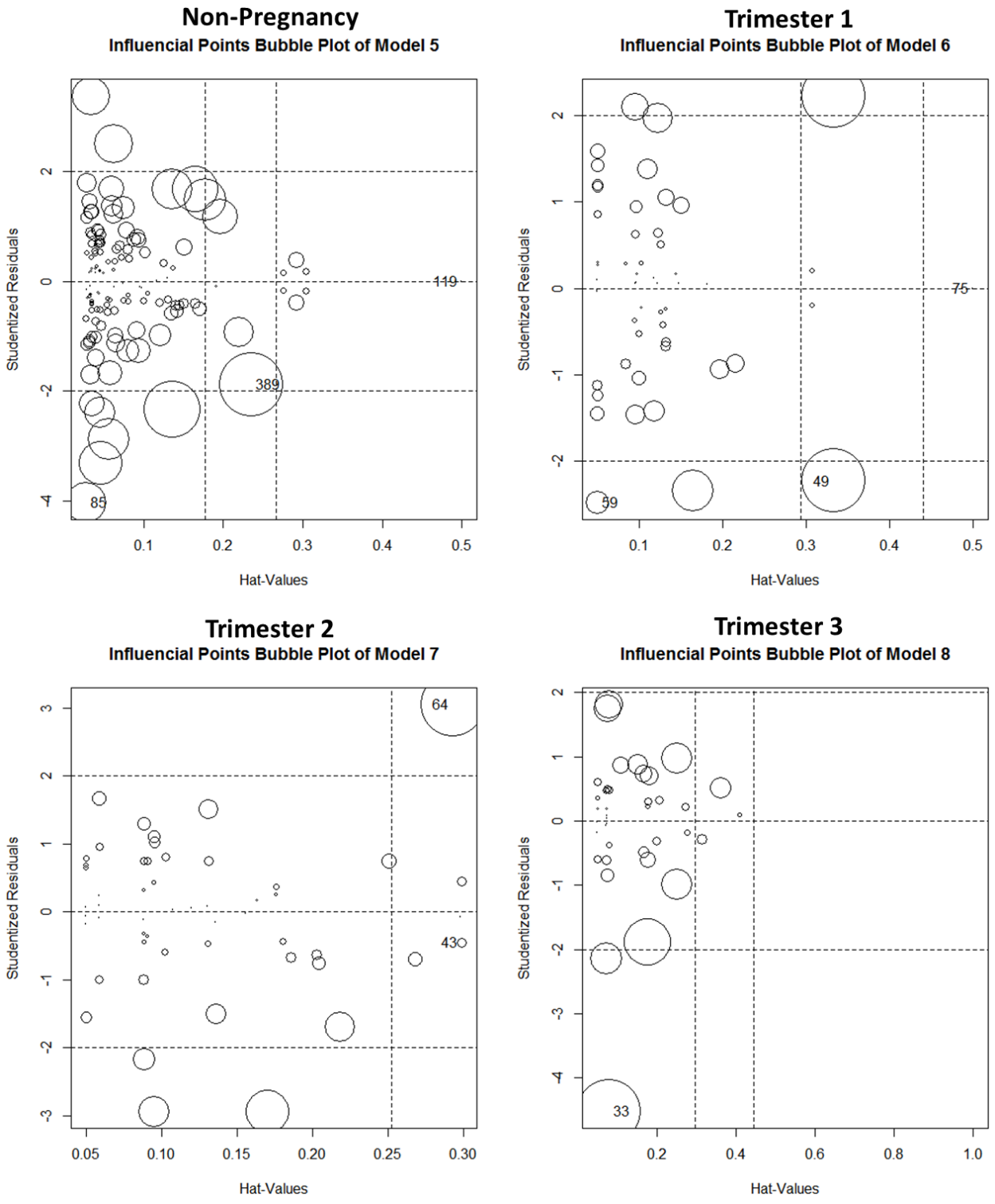

Figure 14: Influential points bubble plot of log (melatonin) regression for four periods

More models were built by cutting off all above influential points, but they were not improved much as expected, so the original models were kept.

3.3.4 Linear regression for cortisol peak value

After considering all factors and all correlations in Section 3.2.1, four regression models of cortisol peak value for non-pregnancy, T1, T2, and T3 were built as below:

```
  Model9<-lm(log(cortisol) ~ playsports * nightshift + paidjob *
workload_weight + slpmed + highbp * prior_pregnancy, data=data_T0)

  Model10<-lm(log(cortisol) ~ slpmed+vitamins+highbp, data=data_T1)

  Model11<-lm(log(cortisol) ~ slp30 + caretoddler + sleepclinic +
workload_weight : nightshift + nightshift+race, data=data_T2)

  Model12<-lm(log(cortisol) ~ race + playsports + paidjob + nightshift +
caretoddler, data=data_T3)
```

Cortisol is considered as a stress hormone because of its connection to the stress response. During non-pregnancy, playing sports, nightshift, paid job, heavy workload at work, or high blood pressure were all related to stress response, further effected cortisol level by increasing the log peak value. Having small dose of sleeping medication increases this outcome, and pregnant women had a protective effect with regard to stress: their cortisol level was found to be lower than the others.

Non-Pregnancy -Model 9

Coefficients:

	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	0.52994	0.26035	2.036	0.042886
playsports1	0.32740	0.10664	3.070	0.002383
playsports2	0.27445	0.09151	2.999	0.002990
nightshift1	0.45330	0.17128	2.646	0.008665
paidjob1	0.81861	0.25390	3.224	0.001436
workload_weight2	0.81781	0.30543	2.678	0.007922
workload_weight3	0.71805	0.36027	1.993	0.047371
slpmed1	0.20683	0.10183	2.031	0.043324
slpmed2	-0.07184	0.13510	-0.532	0.595385
highbp1	0.64027	0.21065	3.039	0.002629
prior_pregnancy1	-0.12250	0.06576	-1.863	0.063698
playsports1:nightshift1	-0.34965	0.22946	-1.524	0.128858
playsports2:nightshift1	-0.92384	0.19178	-4.817	2.56e-06
paidjob1:workload_weight2	-0.91653	0.31620	-2.899	0.004091
paidjob1:workload_weight3	-0.77778	0.37428	-2.078	0.038753
highbp1:prior_pregnancy1	-0.93760	0.26300	-3.565	0.000438

Residual standard error: 0.476 on 243 degrees of freedom
Multiple R-squared: 0.2498, Adjusted R-squared: 0.2035
F-statistic: 5.395 on 15 and 243 DF, p-value: 2.253e-09

Table 15: Regression result of log (cortisol) for non-pregnancy. The “first” level is rolled into the intercept and all subsequent levels have a coefficient that represents their difference from the baseline.

In the first trimester, taking sleeping medication and high blood pressure decreased cortisol peak, while vitamins was found to increase the log cortisol peak by 0.34. This model did not fit well not only because of the low adjusted R^2 value of 0.237, but also because the Q-Q plot showed a lot non-fitted points and not close to normally distributed (Figure# 15).

Trimester 1 -Model 10

```
Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept)  1.16994    0.07356  15.905  < 2e-16
slpmed1     -0.37776    0.15187  -2.487 0.014456
slpmed2     -0.79769    0.30090  -2.651 0.009279
vitamins1    0.34048    0.08753   3.890 0.000177
highbp1     -0.48331    0.30090  -1.606 0.111251

Residual standard error: 0.4126 on 104 degrees of freedom
Multiple R-squared:  0.2653, Adjusted R-squared:  0.237
F-statistic: 9.388 on 4 and 104 DF,  p-value: 1.615e-06
```

Table 16: Regression result of log (cortisol) for trimester 1. The "first" level is rolled into the intercept and all subsequent levels have a coefficient that represents their difference from the baseline.

In the second trimester, women who went to sleep clinic faced increased log cortisol peak by 0.48. Having normal or heavy workload weight interacted with night shift increase the cortisol peak value by 10. The Q-Q plot showed that model 11 is close to normally distributed, even though there were still outliers (Figure 15).

Trimester 2 -Model 11

```
Coefficients:
                              Estimate Std. Error t value Pr(>|t|)
(Intercept)                    1.77259    0.11767  15.064  < 2e-16
slp301                        -0.19080    0.11069  -1.724  0.08816
slp302                        -0.80662    0.17154  -4.702 9.12e-06
caretoddler1                  -0.53188    0.14218  -3.741  0.00032
sleepclinic1                   0.47893    0.26433   1.812  0.07331
nightshift1                   -1.15615    0.52898  -2.186  0.03141
race2                          0.04436    0.14849   0.299  0.76583
race3                          0.40476    0.18115   2.234  0.02790
workload_weight2:nightshift0 -0.16240    0.12986  -1.251  0.21429
workload_weight3:nightshift0 -0.41078    0.18521  -2.218  0.02906
workload_weight2:nightshift1  0.98872    0.52537   1.882  0.06304
workload_weight3:nightshift1  1.09251    0.52491   2.081  0.04021

Residual standard error: 0.4898 on 91 degrees of freedom
Multiple R-squared:  0.4208, Adjusted R-squared:  0.3508
F-statistic:  6.01 on 11 and 91 DF,  p-value: 2.716e-07
```

Table 17: Regression result of log (cortisol) for trimester 2. The "first" level is rolled into the intercept and all subsequent levels have a coefficient that represents their difference from the baseline.

In the third trimester, black/African American and other races had decrease cortisol peak. Women who played sports, had night shift, and took care toddlers were found to have lower cortisol levels. Having a paid job during the third trimester increased pregnant women's stress feeling and further increased their cortisol peak with a p-value equals to 0.026. This model shows good fit with a 0.4805 R^2 and 10.78 F-statistic. The residuals-fitted plot and the Q-Q plot showed it was approximately normally distributed with some outliers as showed in Figure 15.

Trimester 3 -Model 12

```
Coefficients:
             Estimate Std. Error t value Pr(>|t|)
(Intercept)    1.8270     0.2946   6.202 3.94e-08
race2         -0.5243     0.1551  -3.382 0.001207
race3         -0.1884     0.1663  -1.133 0.261127
playsports1   -0.6661     0.2565  -2.597 0.011559
playsports2   -0.5717     0.2314  -2.471 0.016015
paidjob1       0.5054     0.2227   2.270 0.026435
nightshift1   -0.7242     0.1383  -5.237 1.78e-06
caretoddler1  -0.5678     0.1568  -3.622 0.000564
Residual standard error: 0.4077 on 67 degrees of freedom
Multiple R-squared:  0.5296, Adjusted R-squared:  0.4805
F-statistic: 10.78 on 7 and 67 DF,  p-value: 5.141e-09
```

Table 18: Regression result of log (cortisol) for trimester 3. The "first" level is rolled into the intercept and all subsequent levels have a coefficient that represents their difference from the baseline.

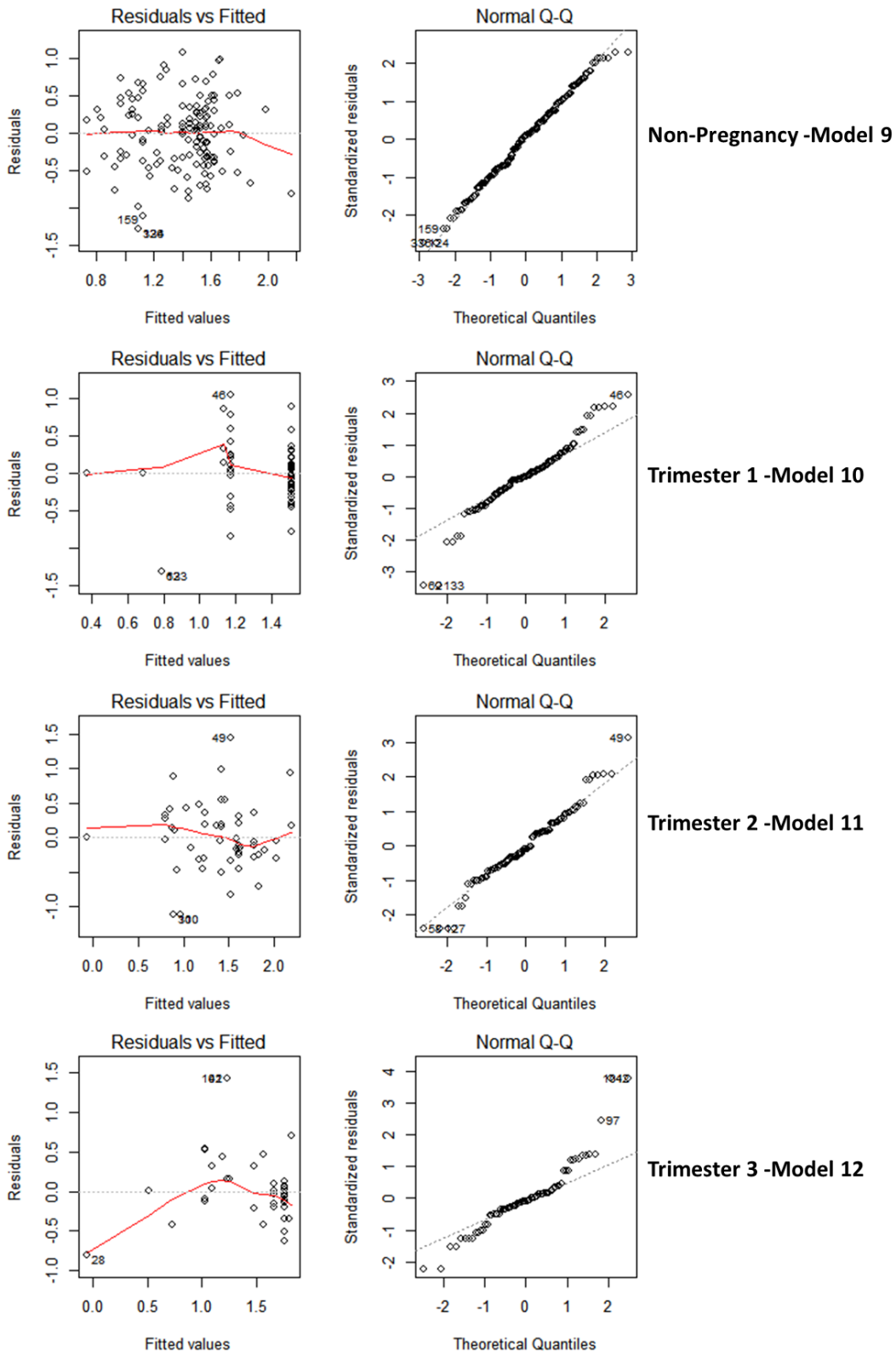

Figure 15: Useful plots of log (cortisol) regression for all periods

All influences for models were showed in Figure 16 with numbers and also quantified below:

Influences for Model 9 (non-pregnancy):

```
##        StudRes        Hat       CookD
## 23   0.2340744 0.30576866 0.001514151
## 43  -1.9152095 0.19781699 0.055919126
## 124 -2.7867193 0.03166389 0.015441088
```

Influences for Model 10 (1st trimester):

```
##      StudRes       Hat        CookD
## 62 -3.656481 0.1503132 4.227538e-01
## 67  0.000000 0.5000000 6.929321e-32
```

Influences for Model 11 (2nd trimester):

```
 ##     StudRes       Hat    CookD
## 49  3.29869 0.1141512 0.105403
## 131     NaN 1.0000000      NaN
```

Influences for Model 12 (3rd trimester):

```
##     StudRes       Hat     CookD
## 42 4.217612 0.1400585 0.2895846
## 97 2.566405 0.5220113 0.8299313
```

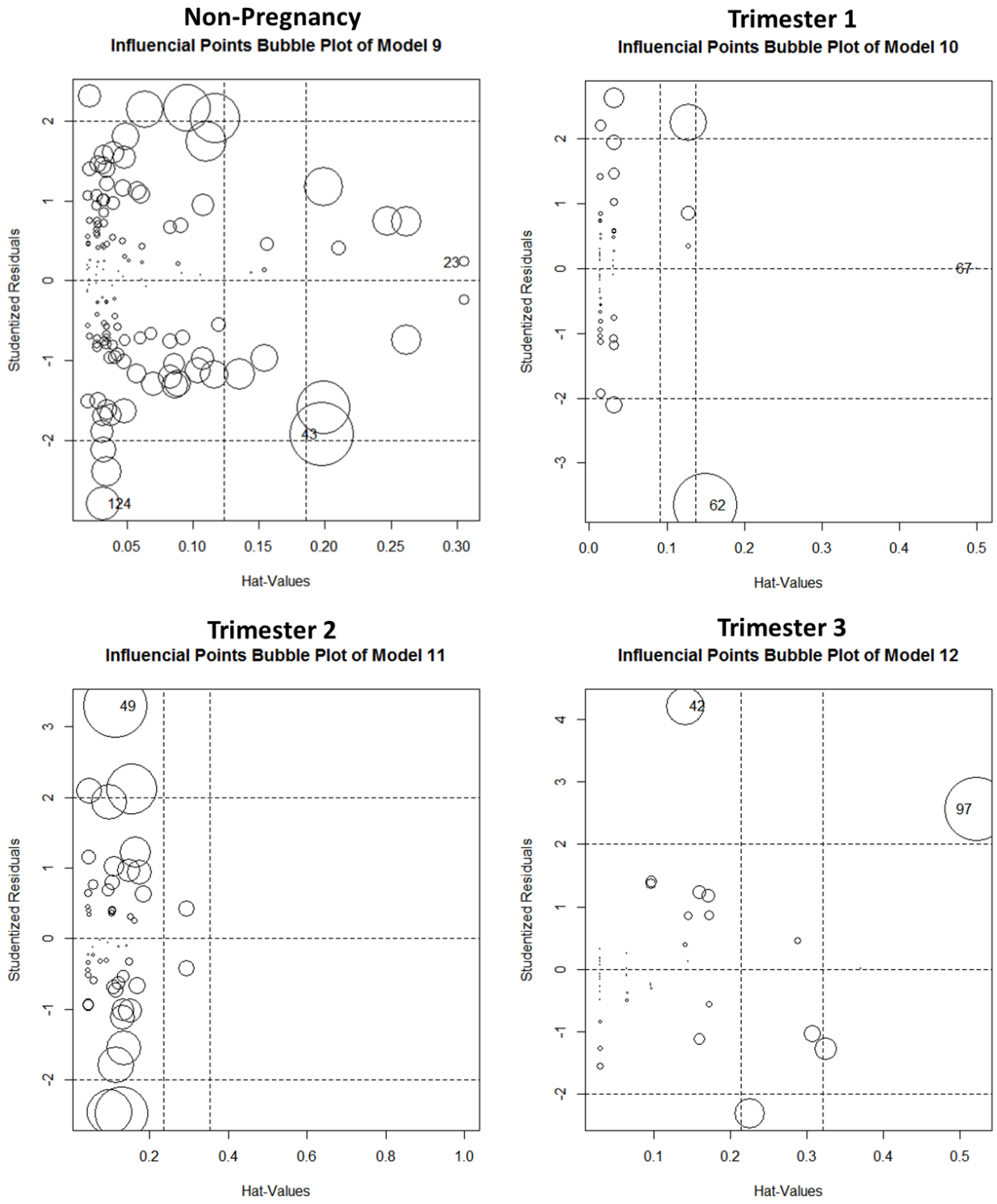

Figure16: Influential points bubble plot of log (cortisol) regression for four periods

More models were built by removing all of the above influential points, but the results were not much different as expected from doing this before with the other hormone, so the original models were kept.

Chapter 4: Discussion

Most of the results found in this study agreed with previous findings in the literature. One difference arose in melatonin secretion. Melatonin peak occurred at about 3 AM in one study [18], while this study showed it happened as early as 2 AM during non-pregnancy, the first trimester, and the third trimester. Interestingly, both workday and free day data in the second trimester had melatonin peak values at about 3 AM. One explanation of this could be the way different hospitals draw participants' blood. In Claustrat's study, blood was collected at 2-hour intervals over a 24-hour period [18]. This study had a random time-picking system for blood draws. The real peak time might be missed in such a case. Another study aimed to examine the effects of night work on salivary melatonin concentration during and subsequent to night work, and thus investigating the mediating role of light [19]. It was found that on work days, night workers showed 15% lower salivary melatonin concentrations compared with day workers. However, on days off, there was no difference observed in melatonin concentration between day and night workers. This explains why this study presented here displayed more variance in weekdays, compared to free days, since data in this study contained both day and night workers.

In previous studies, cortisol levels peak in the early morning (around 8 AM) and reached their lowest level at about 4-6 AM, or three to five hours after the onset of sleep [20]. Mothers have been shown to have higher morning cortisol on days they go to work compared to non-workdays [21]. These findings were the same as what we found in Figure 2 and Figure 3. Because cortisol is produced in the adrenal cortex in response to stress (physical or emotional) and according to natural cycles that tend to correlate to circadian rhythms, cortisol had less peak value in weekdays than in free days because of the chronical stress from work during weekdays [22].

The first and most difficult question was about filtering effective explanatory variables based on theory and model fit. Studies suggest that pregnancy affects sleep in multiple ways. There are hormonal changes, physiologic changes, physical factors, and behavioral changes in pregnant women, all of which may affect her sleep [9]. This study involved as many factors as possible within a more than 713-question long-term survey. It is impossible and unnecessary to analyze all of them. Seventeen related questions and three outcome variables were considered as interesting factors and were selected for models.

We performed ANOVA analysis to understand how our effective factors affect outcome variables from one trimester to another. For sleep midpoint, only a few factors, such as *slp30, nightshift, job_sweat, prior_pregnancy,* had significant effects in non-pregnancy and in the first two trimesters. After getting to the third trimester, this problem became very complex and involved factors in all four SRF, JRF, AWRF and MRF categories. Women in pregnancy did not change many significant factors for melatonin peak between trimesters. For example, setting an alarm clock was important for melatonin peak in all three trimesters. However, using vitamins turned out to be only significant in the third trimester. All factors that gave pregnant women stress were expected to affect cortisol, but this hypothesis was not represented in the data. Only a few factors, including *shiftchange, prior_pregnancy, race, slp30,* and *job_sweat,* affected cortisol peak significantly.

Many of the correlations uncovered in our correlation analysis can be explained by biological common sense. For example, *shiftchange* showed positive correlation with *nightshift*, *workload_weight* had positive correlation with *job_sweat*, *paidjob* had positive correlation with *alarmclock,* and *nder2care* and *caretoddler* had positive correlation with *prior_pregnancy*. Those common correlations happened in all periods. After women got pregnant, more

correlations appeared in the first trimester than baseline, and even more in the second and third trimesters. Two important uses of correlation analysis were to 1) limit the number of factors for regression models and 2) determine interactions. If two factors had large correlation, one of them should be chosen as an explanatory variable. If two factors had small correlation, they either were not chosen or chosen together as an interaction term. However, this just one method for choosing variables, so regression models were built up by trying several combinations of factors after applying this correlation theory, as long as it comports with biomedical reason.

A previous study demonstrated that sleep and wake times had near-Gaussian distributions in a given population [7]. This distribution was predominantly based on differences in an individuals' circadian clock. Similarly, in this study the midpoint sleep time is very close to being normally distributed, not only in baseline, but also in all pregnant periods.

From the presented regression results, variable *weekday* in every period showed about an hour earlier effect on midpoint sleep. Heavy workload weight in pregnancy showed very similar result. This conflicts with the fact that the average American worker reported 5.3 days of difficulty falling asleep, 6.6 days of trouble staying asleep, and 5.0 days of trouble waking up for work in the past month [23]. This conflict could be explained by the fatigue gain in pregnancy, which increased sleep need. In trimester 2, shift change was increased sleep midpoint dramatically. This was not surprising, because shift work was found previously to have strong, acute effects on sleep and the effects seem to linger and also affect days off [24]. Our model had the best fit in the third trimester with relatively narrowed factors and higher R^2 value (0.5473). This was because women right before their delivery had the most uncomfortable situation and least physical movement, so many less effective factors were filtered out by those significant effects.

There were a lot factors and interactions that significantly affected melatonin peak in non-pregnancy period. Job related factors, like paid job, nightshift and heavier workload weight, explained melatonin models in very similar ways to midpoint sleep time. In the second trimester, high blood pressure started to significantly lower melatonin peak value. This finding is similar to a study that demonstrated impaired nocturnal melatonin secretion in hypertensive patients [25]. It was shown in many studies that melatonin levels had a highly significant increase after physical exercise [26]. This explained the factor *playsports*' contribution to models in the second and third trimesters.

Cortisol secretion is related to stress feeling. however, the regression of cortisol peak value was not well explained by using only this simple theory. For instance, high blood pressure in non-pregnancy significantly increased cortisol peak value as shown in other studies [27]. Because smaller correlations appeared in non-pregnancy, it turned out that many factors were responsible for the model fit and some interactions should also be considered. In the first trimester, vitamin use positively affected the cortisol peak value. The effect of vitamins on cortisol is controversial. Vitamin C [28] has been found to increase cortisol in serum while taking vitamin D3 [29] or vitamin E [30] reduced cortisol. Vitamin B12 was shown to have no significant effect on cortisol secretion [31]. Therefore, the types of vitamins taken and durations that these are taken are important considerations for future study.

Studies have also found that increasing exercise positively affected serum cortisol value [32], but in the third trimester of pregnancy we found playing sports decreased cortisol peak during the day. *Paidjob* increased cortisol peak during the third trimester, as expected. However, *nightshift* dramatically dropped cortisol peak, which may be explained by different sleep-awake rhythm for nightshift workers. Surprisingly, black/African Americans had a marked decrease in

cortisol peak value, which has not been reported before. Additional multilevel models could reveal the origins of unexpected results stemming from unpredicted interactions. For example, it was shown previously that cortisol secretion was related to body fat distribution [33, 34].Testing womens' body fat and BMI might reveal the effects of obesity or other dietary issues on cortisol secretion and sleep. Another factor that may also affect melatonin and cortisol would be seasonal changes [35].

In conclusion, factors affecting sleep midpoint and sleep hormones were successfully selected and organized for non-pregnancy and all three pregnant periods. Regression models were built up and tested as well. These results therefore indicate that hormone levels are important effectors of sleep during pregnancy, and point to hormonal dysregulation as a focus of study for pregnancy-related sleep disturbances.

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Appendix

Appendix 1: trans2time function

```
trans2time <- function(x)
{
 time <- as.numeric(unlist(strsplit(as.character(x),":")))
 timestamps<-time[1]+time[2]/60
 if(!is.na(timestamps))
 {
  if( timestamps<12)
  {
   return(timestamps+12)
  }
  else
  {
   return(timestamps-12)
  }
 }
 else
 {
  return(NA)
 }
}
```

Appendix 2: find_maxmaxval function

```
find_maxmaxval <- function(data){
 pid <- unique(data$pid)
 data.mela.max <-matrix(ncol = ncol(data))
 data.cort.max <-matrix(ncol = ncol(data))
 colnames(data.mela.max) <- colnames(data.cort.max) <- colnames(data)
 for(i in 1: length(pid))
 {

  if(!all(is.na(data[data$pid==pid[[i]],2:ncol(data)])))
  {
   temp <- as.data.frame(data[data$pid==pid[[i]],])
   data.mela.max <- rbind(data.mela.max,
temp[temp$melatonin==max(as.data.frame(temp$melatonin), na.rm = F),])
```

```
    data.cort.max <- rbind(data.cort.max,
temp[temp$cortisol==max(as.data.frame(temp$cortisol), na.rm = F), ])
   }
 }
 data.mela.max[data.mela.max$pid=="NULL", ] <- NA
 data.mela.max.2 <- data.mela.max[!is.na(data.mela.max$pid),]
 data.mela.max.2$cortisol <- NULL
 data.mela.max.2$pid <- as.character(data.mela.max.2$pid)
 data.mela.max.2[,2:ncol(data.mela.max.2)] <-
sapply(data.mela.max.2[,2:ncol(data.mela.max.2)], as.numeric)

 data.cort.max[data.cort.max$pid=="NULL", ] <- NA
 data.cort.max.2 <- data.cort.max[!is.na(data.cort.max$pid),]
 data.cort.max.2$melatonin <- NULL
 data.cort.max.2$pid <- as.character(data.cort.max.2$pid)
 data.cort.max.2[,2:ncol(data.cort.max.2)] <- sapply(data.cort.max.2[,2:ncol(data.cort.max.2)],
as.numeric)
 return(list(data.mela.max.2, data.cort.max.2))
}
```